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FLAVONOIDS OF THE GENUS *Bouteloua* (POACEAE) FROM MEXICO

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RESUMEN

Se analizó cualitativamente e identificó el contenido de flavonoides en poblaciones naturales de las especies de *Bouteloua* de México. Se separaron 19 flavonoides en 30 taxa de este género, 12 de ellos se identificaron químicamente. La mayoría resultaron ser derivados glicosilados de las flavonas luteolina, apigenina monoglicosada en las posiciones 5, 6, 7 y 8, y tricina monoglicosada en las posiciones 5 y 7. Se determinaron dos flavonoles Q 3-O rutinosida y Q 3-O glucosida. Los patrones de flavonoides resultantes aportaron un buen carácter taxonómico adicional para la separación de especies. El análisis fenético del contenido de flavonoides dio soporte para concluir que *Bouteloua* merece ser reconocida a nivel genérico y que está compuesta por dos subgéneros: *Bouteloua* y *Chondrosum.*

Palabras clave: *Bouteloua*, Poaceae, Gramineae, flavonoides, quimiosistemática.

ABSTRACT

Flavonoid contents of natural populations of *Bouteloua* species from Mexico were identified and qualitatively analized. Nineteen flavonoids were separated and 12 were chemically identified from 30 taxa of this genus. Most were glycosylated derivatives of the flavones luteolin, apigenin mono-glycosides in the 5, 6, 7 and 8 positions, and tricin mono-glycosides in the 5 and 7 positions. Two flavonols Q 3-O rutinoside and Q 3-O glucoside were determined. Flavonoid patterns provided a good additional taxonomic character for species separation. Phenetic analysis of flavonoid content provided support to conclude that *Bouteloua* deserves a generic level, and it is composed of two subgenera: *Bouteloua* and *Chondrosum*.

Key words: *Bouteloua*, Poaceae, Gramineae, flavonoids, chemosystematics.

INTRODUCTION

The genus *Bouteloua*, a primary component of grasslands and prairies, is ecologically one of the most important grasses in North America. It is one of the most important genera of Poaceae, which has 42 recognized species and 14 varieties. Most of them are xeromorphic living forms in arid lands of the New World. Thirty seven species and 14 varieties occur in Mexico, 20 of these species and 4 varieties are endemisms or occur in restricted areas in Mexico, whereas the rest are extended into the southwestern United States, 6 also into

South America and 3 occur in the West Indies. They are primary components of grasslands in Mexico, being considered the producers of high quality beef in free-range livestock. Many species of *Bouteloua* are significant elements of natural grassland associations and they have a high forage quality for livestock. Species with the widest distribution and economical importance are *B. curtipendula* (side oats grama, banderilla or triguillo), *B. gracilis* (blue grama, navajita), *B. hirsuta* (hairy grama, navajita peluda) and *B. eriopoda* (black grama, navajita negra). At least one of these four species can be found in all grasslands of Mexico.

Previous taxonomic treatments of the genus are conflicting, two subgenera were recognized: *Bouteloua sensu str.* and *Chondrosum* Desv. [Gould, 1979], while Clayton and Renvoize [1986] proposed the division of the genus and raised the rank of these to subgenera. Another author, based on recent molecular studies, has proposed *Bouteloua* as a more inclusive genus, which includes these two taxa as well as the genera of the tribe *Boutelouinae* [Columbus, 1999]. In order to draw evolutionism inferences for this genus from Mexico a systematic study was carried out: *a)* anatomical features in foliar epidermis [Rosales-Carrillo and Herrera-Arrieta, 1996] and culm transversal sections [Siqueiros-Delgado and Herrera-Arrieta, 1996], *b)* morphological characters [Herrera-Arrieta and De la Cerda-Lemus (1997), Esparza-Sandoval and Herrera-Arrieta (1996)] in order to draw evolutionism inferences for the genus. As part of an ongoing survey of the Mexican species sponsored by Comisión Nacional para el Conocimiento y Uso de la Biodiversidad (CONABIO), nearly 3 000 specimens from field collections and herbaria samples were reviewed and scored to produce a data base. The objective of the present study is to examine the flavonoid characters of the taxa within *Bouteloua* in order to clarify species boundaries and determine relationships within the genus. The species and populations included in the study are listed in Table 1 and their approximate locations shown in fig.1 (species not found in field collections were excluded from the analysis).

MATERIAL AND METHODS

A survey of the flavonoid content in the species of the genus *Bouteloua* was conducted over 148 populations. Bulk samples (ca. 50 g) of ground, air-dried plant material were taken from field collections of plants at the flowering stage. The powder was extracted in 80% MeOH and then 50% MeOH for 24 h each. The extracts were combined, concentrated and surveyed for flavonoid composition using standard 2-D paper chromatography (BAW and 15% HOAc). At least one population from each species was subjected to exhaustive analysis. In all cases the population chosen contained the greatest number of flavonoids. In a few instances more than one population had to be extracted so that all of the flavonoids could be analyzed. In the exhaustive analysis, the concentrated extracts were partitioned successively with chloroform and ethyl acetate. Flavonoids from all fractions were separated and purified using standard methods of paper and thin-layer chromatography. Most flavonoids were found in the ethyl acetate fraction. Identification of flavonoids was accomplished using standard spectral and hydrolytic techniques [Mabry *et al*. (1970), Markham (1982)]. Rf values were determined on Whatmann # 1 paper in four solvent systems, 4:1:5 (butanol: acetic acid: H_2O-BAW), H_2O, 15% acetic acid, 80% phenol. Unknown cases were co-chromatographed

against rutin (quercetin 3-O rutinoside) standard. Hydrolyzed sugars were identified by co-chromatography against standard sugars on cellulose TLC (Polygram Cel 300) in 18:18:3.5:10.5 (pyridine: ethyl acetate: acetic acid: H_2O) followed by staining with aniline diphenylamine phosphate. Suspected C-glycosyl flavones were chromatographed with 15% HOAc on cellulose plates (TLC), and sprayed with NA reagent [Markham, 1982] to check for Wessley-Moser rearrangement products [Mabry *et al.* (1970), Markham (1982)].

An overall similarity analysis, using Jaccard s' coefficient to compute similarities, was performed on the data set of Table 3, followed by a cluster analysis using unweighted pair-group mathematical average (UPGMA) of the similarity matrix. Analyses were performed using Multivariate Statistical Package [Kovach, 1987].

RESULTS AND DISCUSSION

Species names and approximate locations of the 148 population samples (Table 1) are shown in fig. 1. For summarizing species relationships, phenetic analysis of the 19 compounds, qualitatively identified in these populations was performed using the unweighed pair group method of averaging (UPGMA), to interpret variation in flavonoid expression. Vouchers were deposited at CIIDIR herbarium. Found compounds are shown in Table 2.

Nineteen flavonoid compounds were isolated from whole plant extracts and 13 of them identified (table 2). Eleven of these are glycosylated derivatives of flavones luteolin, apigenin and tricin. Two other are glycosylated flavonols of quercetin. Six different compounds were detected but have not been fully characterized, mainly because of low concentration found in plants, these compounds does not appear at Table 2 and are mentioned in Table 3 as compounds: 14, 15, 16, 17, 18 and 19. The flavonoid profiles of the species are summarized in Table 3, where the presence of each compound was registered.

The number after the species name refers to the number of populations freshly collected and analyzed for each species. Little intraspecific variation was detected as indicated in Table 3, resulting in generally uniform profiles within a taxon, which therefore, are taxonomically useful. Generated data can be taken as an indicator of the variability process within the genus. A more complete population sampling is needed for an interspecific analysis.

The results of the cluster analysis based on the 19 compounds listed in tables 2 and 3 are presented in the form of a dendrogram (fig. 2). Profiles of the taxa are all very similar, with most of the variation occurring in number and type of sugar attached to the molecule. In general, the genus is relatively homogenous, lacking substantial differences among the taxa. In the case of varieties, flavonoid profiles were consistently similar within species. The dendrogram (fig. 2) suggests that the 36 analysed taxa seem to be part of a natural group.

As can be seen from fig. 2, some groups inside an homogeneous tree are perceived, based on flavonoid data, with all species having divergent geographic distribution. The chemical groupings do not support perceived relationships based on morphological criteria [Herrera-Arrieta and De la Cerda-Lemus, 1997]. Modern taxonomic arrangements of *Bouteloua* (*sensu lato*) have been separated into two

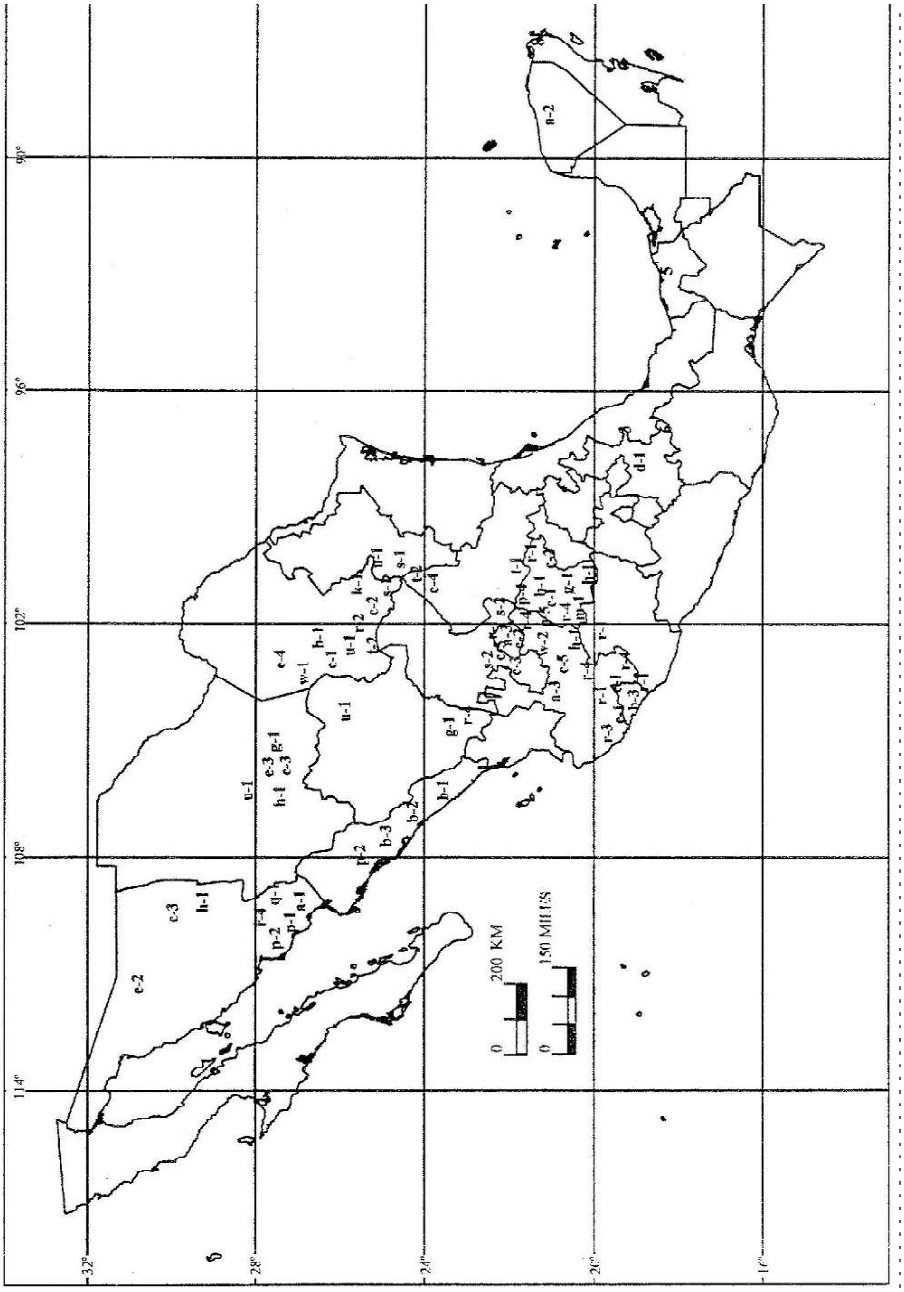

Fig. 1. Collection site locations for *Bouteloua* species used in flavonoid analyses. Letter-number codes refer to species in Table 3.

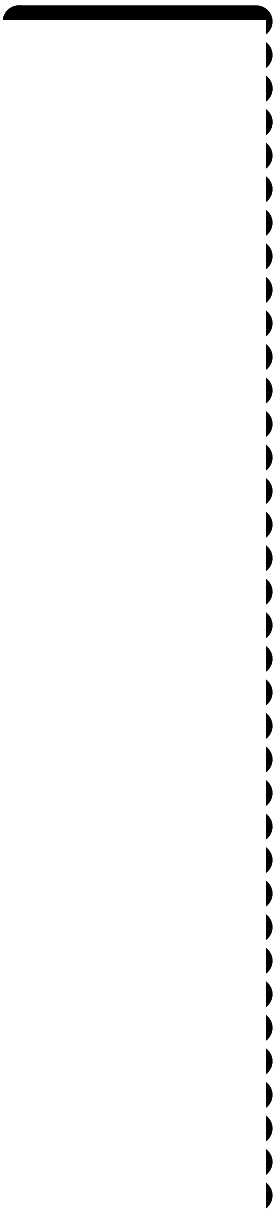

Fig. 2. UPGMA Cluster analysis of flavonoid characters (Table 3) for 36 taxa of *Bouteloua*. Correlation.

genera *Bouteloua* and *Chondrosum*, based only in features of the inflorescence [Clayton and Renvoize, 1986]. Flavonoid profiles from the species of the genus do not provide support for this separation, because the four chemically related groups contain species from both proposed genera.

The number of flavonoids found within the species of *Bouteloua* ranges from 8 to 13 flavonoids per species. Species with the higher number of flavonoids are: *B. barbata* var. *barbata, B. hirsuta* var. *hirsuta, B. uniflora* var. *coahuilensis, B. parryi* var. *parryi* and *B. williamsii*; however these taxa are not morphologically similar, and they do not share physiographic affinities, in fact, some species are primary components of the grasslands (*B. hirsuta, B. uniflora, B. williamsii*), and the remainder are from disturbed places (*B. barbata, B. parryi*). Geographic distribution of these five species is also different, *B. barbata, B. hirsuta, B. uniflora* are species of very wide distribution, whereas *B. parryi* and *B. williamsii* belong to restrict places. *B. barbata* and *B. parryi* are annual species that grow between 50 and 2550 m, where they are sympatric. They share habitat preferences and complete their life cycle in about four weeks after a summer rain. Because of the short life cycle of annual plants, presumably they would have less time to form flavonoids than perennial species; however, annual species produce as many flavonoids as three of perennial species and a greater number of flavonoids than in the 31 other analyzed species. Groups of species containing a lower concentration of flavonoids are *B. eludens, B. media, B. ramosa, B. scorpioides* and *B. triaena*, containing 9 flavonoids, whereas *B. eriopoda* and *B. karwinskii* have shown only 8. All are perennial species, morphologically dissimilar, growing at elevations among 500 to 2500. *Bouteloua karwinskii, B. scorpioides* and *B. ramosa*, belong to saline soils of desertic areas (Chihuahuan Desert). *Bouteloua eludens* and *B. eriopoda*, belong to restricted areas of the Mexican oak and pine forest at NW Mexico. *Bouteloua media* and *B. triaena* occur in the deep soils in the agricultural region at central Mexico.

The patterns of flavonoid variation in the taxa of *Bouteloua* provided supplementary characters to support the delimitation of the recognized species based on morphological features. From morphological evidence [Herrera-Arrieta and De la Cerda-Lemus, 1997] it would appear that the genus is composed of a very closely related group of species. However, flavonoid evolution has occurred at a different rate than morphological evolution. This is not surprising, since enough cases have been documented where chemical variability has more rapidly evolved than morphology or viceversa [Crawford and Mabry (1978), Giannasi, 1975].

The results of this study could provide bases to recognize all the species of *Bouteloua* as a putative group.

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Table 1. Species of ***Bouteloua*** collected for this study.

Table 1. Continuation.

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Table 2. Chromatographic, spectral and *Rf* data for Flavonoid Glycosides of *Bouteloua* from Mexico.

100 % MeOH	NaOMe	AlCl3	AlCl3/HCl	NaOAC	H3BO3	*Rf* X 100	Colours +

* Compounds: A, apigenin; L, luteolin; Q, quercetin; T, tricin; (Compounds 14-19 not fully identified). Colour key+: Bl, blue; Fl, fluorescent; O, orange; P, purple; Y, yellow. NA, Naturstoffeagens A in MeOH.

Table 3. Flavonoids in the species of *Bouteloua* from Mexico.

Compounds code:	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
Taxon:																			
a-1 *B. alamosana* (1)	-	+	+	+	-	+	+	+	+	-	+	+	+	+	-	-	-	-	-
a-2 *B. americana* (1)	+	-	+	+	-	-	+	-	+	+	+	+	-	-	+	+	-	-	+
a-3 *B. aristidoides* (2)	+	+	+	-	-	+	-	-	-	+	+	+	+	-	+	+	-	+	-
b-1 *B. barbata* var. *barbata* (7)	+	+	+	+	-	-	+	-	+	+	+	+	+	+	+	-	-	-	-
b-2 *B. barbata* var. *rothrockii*(2)	+	+	+	+	-	-	+	-	+	+	+	+	-	+	+	-	-	-	+
b-3 *B. barbata* var. *sonorae* (3)	+	+	+	+	-	-	+	-	+	+	+	+	-	+	+	-	-	+	-
c-1 *B. curtip.* var. *curtip.* (2)	-	+	+	+	-	-	+	+	-	+	+	+	+	-	-	+	+	-	-
c-2 *B. curtip.* var.*caespitosa* (11)	-	+	+	+	-	-	+	+	-	+	+	+	+	-	-	+	+	-	-
c-3 B. *curtipen.* var. *tenuis* (3)	-	+	+	+	-	-	+	+	-	+	+	+	+	-	-	+	+	-	-
c-4 *B. chasei* (3)	-	+	-	+	+	+	-	+	+	+	-	-	-	+	+	-	-	-	+
c-5 *B. chondrosioides* (9)	+	+	+	+	-	-	+	-	+	+	+	+	+	+	+	-	-	-	-
d-1 *B. distans* (1)	-	-	+	+	-	+	+	-	+	+	+	+	+	+	+	-	-	-	-
e-1 *B. elata* (3)	+	+	+	-	-	-	+	-	+	+	+	-	+	+	+	-	-	-	-
e-2 *B. eludens* (1)	+	+	+	-	-	+	-	-	-	-	+	+	+	+	-	-	-	+	-
e-3 *B. eriopoda* (1)	-	-	+	+	-	-	+	+	+	-	-	+	+	+	-	-	-	-	-
e-4 *B. eriostachya* (1)	-	+	+	-	+	+	-	+	+	+	+	+	+	-	-	-	-	-	-
g-1 *B. gracilis* (16)	+	-	+	+	-	-	+	-	+	+	+	+	+	+	+	-	-	-	-
h-1 *B. hirsuta* (15)	+	+	+	+	+	-	+	-	+	+	+	+	+	-	+	-	-	+	-
k-1 *B. karwinskii* (1)	-	+	-	+	+	+	-	+	+	+	-	-	-	-	-	-	-	-	+
m-1 *B. media* (1)	-	-	+	+	-	-	+	+	-	+	-	+	+	+	-	-	+	-	-
p-1 *B. parryi* var. *parryi* (3)	+	+	+	+	+	-	+	-	+	+	+	+	+	-	+	-	-	+	-
p-2 *B. parryi* var. *gentryi* (3)	-	+	+	+	+	-	+	-	+	+	+	+	-	-	+	-	-	+	-
p-3 *B. pedicellata* (1)	+	-	+	+	-	-	+	-	+	+	+	+	+	+	+	-	-	-	-
p-4 *B. purpurea* (1)	+	+	+	-	-	+	-	-	-	+	+	+	+	+	-	-	-	+	-
q-1 *B. quiriegoensis* (1)	+	-	+	-	-	-	-	+	+	+	+	+	+	+	-	-	-	-	+
r-1 *B. radicosa* (8)	-	-	+	+	-	+	+	-	+	+	+	+	+	+	+	-	-	-	-
r-2 *B. ramosa* (2)	-	+	-	+	+	+	-	+	+	+	-	+	+	-	-	-	-	-	-
r-3 *B. reflexa* (1)	+	+	+	-	-	-	+	-	+	+	+	+	+	+	+	-	-	-	-
r-4 *B. repens* (22)	-	+	+	+	-	-	+	-	+	+	+	+	+	-	-	+	-	-	-
s-1 *B. scorpioides* (4)	+	+	-	+	-	-	+	-	+	+	+	+	-	+	-	-	-	-	-
s-2 *B. simplex* (3)	+	-	+	-	+	-	+	-	+	+	+	+	-	+	+	-	-	-	-
t-1 *B. triaena* (1)	-	+	+	-	+	-	+	+	-	+	+	+	+	-	-	-	-	-	-
t-2 *B. trifida* (4)	+	+	+	+	-	-	+	-	+	+	+	+	+	+	-	+	-	-	-
u-1 *B. uniflora* var.*coahuil.* (7)	+	+	+	+	-	-	+	-	+	+	+	-	+	+	+	+	+	-	-
w-1 *B. warnockii* (1)	+	+	+	-	-	+	-	-	-	+	+	+	+	+	-	+	-	+	-
w-2 *B. williamsii* (1)	-	+	+	+	+	+	-	+	+	+	+	-	+	+	+	-	+	-	-

Note: number of populations examined is given in parentheses. Compounds are in Table 2.