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Variability of the Foliar Phenol Profiles of the *Agave victoriae-reginae* Complex (Agavaceae)

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Abstract: The foliar phenol profiles of eight populations of the *Agave victoriae-reginae* complex were determined by high performance liquid chromatography with diode array detection in order to assess the variability of those compounds across the natural distribution of the complex, and to determine their taxonomical significance. With comparative aims *Agave lechuguilla* and *A. striata* were analyzed in the same manner. A total of 81 phenolic compounds were detected, comprising 18 phenolic acids, 51 flavonoids (26 flavonols, 19 dihydrovonoids, and six flavones), and 12 non identified phenols. Each population of the *A. victoriae-reginae* complex showed a different foliar phenol composition, and *A. pintilla*, the most recently described species of the group, could be distinguished by four compounds not shared with any other analyzed population. Phenolic acids were detected in all taxa. However, a higher proportion of those compounds was found in the *A. victoriae-reginae* complex, while a predominance of flavonols (quercetin-3-*O*-glycosides) was found in *A. striata*, and a codominance of dihydroflavonoids and flavonols in *A. lechuguilla*, allowing to distinguish species-specific profiles. Differences in the contents of some phenolic compounds among those three species and within the *A. victoriae-reginae* complex were also found. Our results sustain the proposal of considering *A. victoriae-reginae* as a species complex and the separation of *A. pintilla* as an independent species. The results also reveal the foliar phenol patterns as valuable chemical markers in *Agave*.
Key words: *Agave lechuguilla*, *Agave striata*, *Agave victoriae-reginae*, chemical variability, phenol profiles.

Resumen: Se determinaron los perfiles fenólicos foliares de ocho poblaciones del complejo *Agave victoriae-reginae* por medio de cromatografía líquida de alta resolución con detector de arreglo de diodos para evaluar la variabilidad de esos compuestos a través de la distribución natural del complejo, y para determinar su valor taxonómico. Con fines comparativos *Agave lechuguilla* y *Agave striata* se analizaron de la misma manera. Se encontró un total de 81 compuestos fenólicos, que incluyeron 18 ácidos fenólicos, 51 flavonoides (26 flavonoles, 19 dihidroflavonoides y seis flavonas), además de 12 compuestos fenólicos no identificados. Cada población del complejo *A. victoriae-reginae* presentó una composición fenólica foliar diferente, y *A. pintilla*, la especie más recientemente descrita del grupo, se pudo distinguir por cuatro compuestos no compartidos con ninguna otra población analizada. En todos los taxa se detectaron ácidos fenólicos; sin embargo, una predominancia de estos compuestos se encontró en el complejo *A. victoriae-reginae*, mientras que en *A. striata* se encontró predominancia de flavonoles (derivados de quercetina), y en *A. lechuguilla* una codominancia de dihidroflavonoides y flavonoles, lo que permitió discernir perfiles especie-específicos. Diferencias en los contenidos de algunos compuestos fenólicos entre estas tres especies y dentro del complejo *A. victoriae-reginae* también fueron encontradas. Los resultados son consistentes con la propuesta de considerar a *A. victoriae-reginae* como un complejo de especies y con la separación de *A. pintilla* como una especie independiente. Estos resultados revelan a los patrones fenólicos foliares como marcadores químicos valiosos en *Agave*.
Palabras clave: *Agave lechuguilla*, *Agave striata*, *Agave victoriae-reginae*, perfiles fenólicos, variabilidad química.

Agave victoriae-reginae T.Moore is one of the about 166 (Good-Ávila *et al*., 2006) to 200 (García-Mendoza, 2002) species of *Agave*. It is one of the most important ornamental species of the genus, belonging to subgenus *Littaea* (Gentry, 1982). Because its popularity as ornamental, some of its populations have become endangered due to illegal collections, and the Mexican government has listed the species as endangered to protect it (SEMARNAT, 2010). It has

been considered as endemic to the Chihuahuan Desert of Mexico, with populations occurring in western Nuevo León, central Coahuila, and northeastern Durango (Eguiarte *et al*., 1999; Martínez-Palacios *et al*., 1999, 2003), as well as in southern Durango (González-Elizondo *et al*., 2009), outside the limits of the Chihuahuan Desert. High levels of genetic diversity within populations and of differentiation among populations of *Agave victoriae-reginae* were found by Martínez-Palacios *et al*. (1999), who reported that the levels of differentiation among some populations are comparable to those observed among subspecies or even species.

A recent taxonomic revision (González-Elizondo *et al*., 2011) reveals that what has been considered as *A. victoriae-reginae* represents a complex of three species, one with two subspecies: (1a) *Agave victoriae-reginae* subsp. *victoriae-reginae* (western Nuevo León and eastern Coahuila); (1b) *A. victoriae-reginae* subsp. s*wobodae* J.J.Halda (southern Coahuila and northeastern Durango); (2) *Agave nickelsiae* Goss. ex Rol.-Goss. (microendemic to southeastern Coahuila); and (3) *Agave pintilla* S.González, M.González & L.Reséndiz, a newly described species (southeastern Durango). References to this complex from now on in this paper are given as *A. victoriae-reginae* s.l., whereas references to *A. victoriae-reginae* as a species are given without "s.l." or, when both cases are contrasted, as *A. victoriae-reginae* s. str.

Flavonoid profiles have been reported as worthy chemical markers with a species-specific tendency (Campos, 1997; Fiasson *et al*., 1997; Almaraz-Abarca *et al*., 2004; Almaraz-Abarca *et al*., 2006). In spite of the many taxonomic controversies about the delimitation of many species of *Agave* (Gentry, 1982; González-Elizondo *et al*., 2009), efforts focused in determining the taxonomic significance of the flavonoid profiles in this genus have been scarce. *Agave americana* (Parmar *et al*., 1992; Subramanian and Nair, 1970; Tinto *et al*., 2005), and *A. barbadensis* (Tinto *et al*., 2005) are among the few species of *Agave* studied for the flavonoid composition, although with no taxonomic aims. For those species, complex flavanoids were reported. One study focused on the taxonomic significance of the phenol profiles in *Agave* (Almaraz-Abarca *et al*., 2009), pointed out that foliar flavonoid profiles are valuable markers to discriminate among *Agave asperrima*, *A. durangensis*, *A. shrevei* subsp. *shrevei*, *A. shrevei* subsp. *matapensis*, and *A. wocomahi*.

In the present study, foliar flavonoid profiles of eight populations representing the total geographical distribution of *Agave victoriae-reginae* s.l. were obtained by high performance liquid chromatography with diode array detection (HPLC/DAD) in order to establish the variability among populations and the significance of these markers to support taxonomical proposals based on morphological traits. *Agave lechuguilla* Torr. and *A. striata* Zucc. were analyzed in the same manner as references and for identifying some accumulation tendencies of phenolic compounds in species of subgenus *Littaea*.

Materials and methods

Plant material. Foliar tissue of adult plants of 64 individuals from eight populations assumed as *Agave victoriae-reginae*, covering its natural distribution, was analyzed for their phenol composition by HPLC/DAD. With comparative purposes, ten individuals of *Agave lechuguilla* (series 2000) and an equal number of individuals of *Agave striata* (series 1000) were collected and analyzed in the same manner. Voucher specimens for each population sampled were deposited at Herbarium CIIDIR. The description of each sampling site and the number of individuals sampled for population and species are shown in Table 1.

Phenol extraction. Each sample was individually analyzed. Five grams of foliar dried grinded tissue were extracted by maceration for 24 h, in 25 mL 60% methanol (v/v), in darkness and at room temperature. The extracts were centrifuged (8,500 rpm) for 10 min at room temperature. The supernatants were separated and the pellets reextracted in 25 mL 60% methanol (v/v) by maceration for 3 h. The extracts were centrifuged at the same conditions. Similar supernatants were brought together to form the total extracts; these were concentrated and fractionated with ethyl acetate before taking them to dryness under vacuum. Each organic fraction was then dissolved in 3 mL methanol; aliquots were taken to be used in the HPLC/DAD analyses.

HPLC/DAD analysis. The individual HPLC/DAD phenol profiles were obtained according to the method described by Campos (1997). Extracts (20 μL) were analyzed on a Perkin Elmer Series 200 HPLC system and Perkin Elmer Brownlee Analytical C18 column (4.6 × 250 mm, 5 μm), by an acidified acetonitrile-water gradient. The flow rate was 0.8 mL/min. Standard chromatograms were plotted at 280 and 340 nm. Spectral data for all peaks were accumulated in the range 200-400 nm using a diode-array detector (DAD) (Perkin Elmer Series 200). The structural information was obtained by direct comparison of retention times and UV spectra of the compounds found with those of standards, and according to the compilations done by Mabry *et al*. (1970) and Campos and Markham (2007). The contents of eight selected compounds, according to their presence only in both *A. lechuguilla* and *A. striata* (compounds **4** and **33**), only in *A. victoriae-reginae* from El Mezquital (compounds **5** and **37**), and only in most populations of *A. victoriae-reginae* s.l. (compounds **8**, **11**, **29**, and **48**) were estimated by calibration curves (5-100 μg mL^{-1} vs. peak area) constructed by linear regression via HPLC (peak areas) obtained from quercetin-3-*O*-rhamnoside (intercept = -0.00121, slope = 0.05849, r = 0.9963) for quercetin derivatives, and from gallic acid (intercept = 0.0184, slope = 0.0894, r = 0.9963) for derivatives of phenolic acids. The contents were expressed in μg g^{-1} dry weight. They were estimated and compared for samples co-

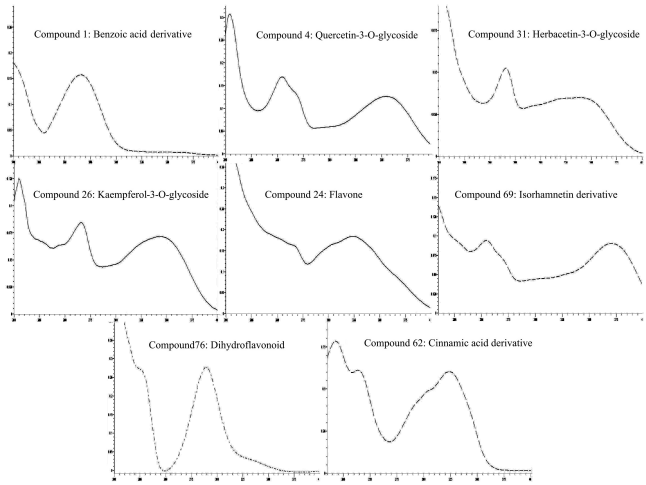

Figure 1. UV-spectra of compounds representing the classes of phenols detected in the foliar tissues of *Agave victoriae-reginae* s.l., *Agave lechuguilla*, and *Agave striata*.

llected in April-May (the population of Viesca was excluded because it was collected in August), and in April-May and August only for the samples of *A. victoriae-reginae* from El Mezquital.

Data analysis. The individual phenol profiles were made up of all compounds present in the respective HPLC/DAD chromatograms. Each compound was treated as a single chemical character. A binary matrix coded by 1 (presence) or 0 (absence) formed by all individual samples vs. all resolved compounds (84 individuals *vs*. 81 compounds) was analyzed using Principal Components Analysis (PCA) from Past 1.43 (Hammer *et al*., 2001).

Results

Foliar phenol composition. Eighty one compounds were detected for the 84 analyzed individuals. Their retention times

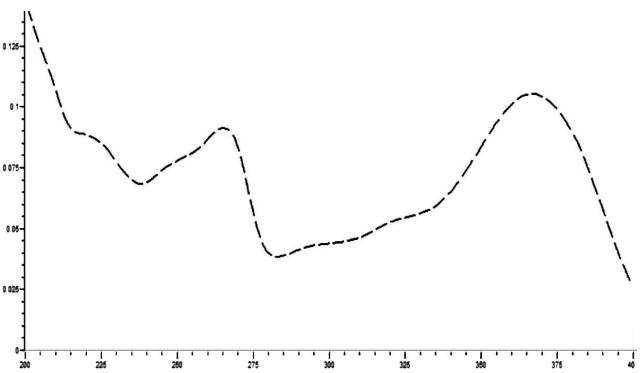

Figure 2. UV-spectrum of free kaempferol detected in *Agave victoriae-reginae* of Bustamante, Nuevo León, Mexico.

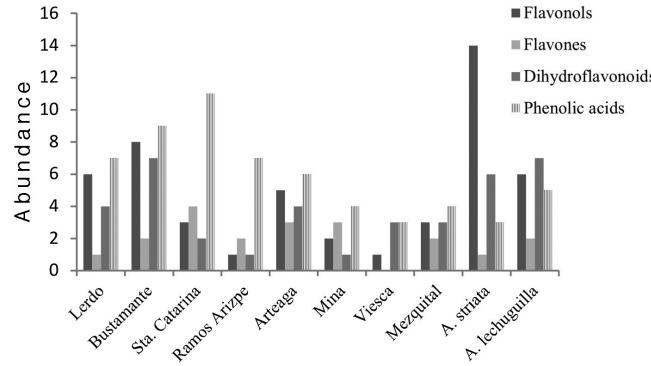

Figure 3. Abundance of the different classes of phenols in the foliar tissues of *Agave victoriae-reginae* s.l., *A. lechuguilla*, and *A. striata*.

and spectral data are shown in Table 2. Flavonoids (51 compounds) and phenolic acids (18 compounds) were the major phenols detected in the foliar tissue of *Agave victoriae-reginae* s.l., *A. lechuguilla*, and *A. striata*. Among the phenolic acids, nine were identified as benzoic acid derivatives and nine as cinnamic acid derivatives. Flavonols (26 compounds), flavones (6 compounds), and dihydroflavonoids (19 compounds) were the classes of flavonoids identified. Figure 1 shows the UV-spectra of compounds representing

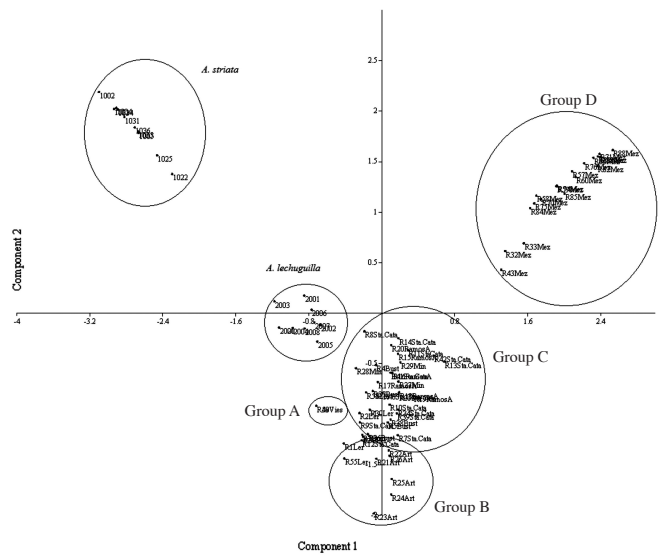

Figure 4. Results of Principal Components Analysis comparing foliar flavonoid profiles for 84 samples of eight populations of the *Agave victoriae-reginae* complex s.l., one population of *A. striata*, and one of *A. lechuguilla*. The groups for the *A. victoriae-reginae* complex are: (A) population of Viesca; (B): populations of Arteaga, some individuals of Bustamante, and Lerdo; (C): populations of Mina, Santa Catarina, Ramos Arizpe, and some indiviudals of Bustamante; (D): population of El Mezquital.

Table 1. Collection sites for *Agave victoriae-reginae* s.l., *Agave striata*, and *Agave lechuguilla*. N: number of analyzed individuals

Species	Location	N	Latitude (N)	Longitude (W)	Altitude (m)	Sample	Date
A. victoriae-reginae	Lerdo, Durango	7	25° 16′	103° 45′	1,300	R1, R2, R30, R52-R55	April/2009 May/2009 August/2009
A. victoriae-reginae	Bustamante, Nuevo León	6	26° 34′	100° 39′	600	R3-R5, R36-R38	May/2009
A. victoriae-reginae	Sta. Catarina, Nuevo León	14	25° 29′ 33″	100° 23′ 7″	1,443	R7-R14, R34, R35, R39-R42	May/2009
A. victoriae-reginae	Ramos Arizpe, Coahuila	6	25° 30′ 38″	100° 54′ 4″	1,502	R15-R20	May/2009
A. victoriae-reginae	Arteaga, Coahuila	6	25° 28′ 31″	100° 51′ 38″	1,688	R21-R26	May/2009
A. victoriae-reginae	Mina, Nuevo León	3	25° 57′ 56″	100° 36′ 7″	645	R27-R29	May/2009
A. victoriae-reginae	El Mezquital, Durango	19	23° 30′ 36″	104° 23′ 42″	1,453	R32, R33, R43, R57-R60, R70, R71, R74-R76, R82-R88	May/2009 June/2009 October/2009
A. victoriae-reginae	Viesca, Coahuila	3	25° 11′ 24″	102° 38′ 59″	1,292	R48-R50	August/2009
A. striata	Lerdo, Durango	10	25° 15′ 39″	103° 4′ 29″	1,311	1002, 1004, 1005, 1021, 1022, 1025, 1031, 1034-1036	April/2009
A. lechuguilla	Lerdo, Durango	10	25° 16′ 17″	103° 45′ 4″	1,303	2008, 2021	April/2009

the different classes of phenols detected in the foliar tissue of *A. victoriae-reginae* s.l., *A. lechuguilla*, and *A. striata*.

Kaempferol (a flavonol aglycone, compound **68**) was detected in *Agave victoriae-reginae* of Bustamante, Nuevo León; its UV-spectrum is shown in Figure 2. The foliar phenol profiles of the different *A. victoriae-reginae* s.l. populations, *A. striata*, and *A. lechuguilla* are shown in Table 3.

Taxonomic significance. The abundance of each type of phenolic compound found in the foliar tissue of *Agave victoriae-reginae* s.l., *A. striata*, and *A. lechuguilla* are shown in Figure 3. Variations in the accumulation levels of compounds can be observed in the different taxa. The levels of eight compounds (**4**, **5**, **8**, **11**, **29**, **33**, **37**, and **48**) present in the taxa analyzed are displayed in Table 4. Some variations among taxa and between two seasons in the same taxon (*A. victoriae-reginae* s.l. from El Mezquital) are observed.

The PCA based in a matrix constructed with all the compounds resolved in the HPLC/DAD chromatograms vs. all the individuals analyzed, revealed the separation of *Agave striata* from *A. lechuguilla* and *A. victoriae-reginae* s.l. (Figure 4). The separation of *A. lechuguilla*, *A. victoriae-reginae* s.l. from El Mezquital, and all other populations of *A. victoriae-reginae* s.l., is observed in the same Figure 4, agreeing with the fact that they are independent species.

Discussion

Foliar phenol composition. In order to obtain fingerprinting chromatograms, the HPLC analysis were done with the unhydrolyzed extracts. The HPLC/DAD is considered as a highly effective technique for the separation and for obtaining structural information of phenolic acids and flavonoids (Markham and Bloor, 1998).

The presence of kaempferol (compound **68**) in *Agave victoriae-reginae* of Bustamante, Nuevo León, one of the most arid sites among the studied here, and the report about flavonoid aglycones being most often produced and accumulated externally in plants from arid and semiarid habitats (Wollenweber, 1994) suggest that the species here analyzed may synthesize more flavonoid aglycones, but they could not be detected because polar solvents were used in the extract preparations, which preferentially dissolve flavonoid glycosides. Even so, the identification of 25 flavonol glycosides in the taxa of *Agave* here analyzed indicate that these species of plants from arid and semiarid habitats can

Table 2. Chromatographic and spectral data for the foliar phenol compounds of *A. victoriae-reginae* s.l., *A. striata*, and *A. lechuguilla*.

Compound	Type of compound	Retention Time (min)	λ_{max} · (nm)
1	Benzoic acid derivative	23.104 ± 0.118	266
2	Flavonol	23.027 ± 0.110	225sh, 256, 268, 350
3	Cinnamic acid derivative	25.010 ± 0.000	259sh, 287
4	Quercetin-3-*O*-glycoside	27.224 ± 0.195	255, 274sh, 355
5	Unidentified	27.181 ± 0.106	226sh, 254, 270sh, 356
6	Quercetin-3-*O*-glycoside	28.045 ± 0.320	256, 269sh, 356
7	Flavonol	28.868 ± 0.132	252, 270sh, 358
8	Benzoic acid derivative	29.513 ± 0.044	248
9	Dihydroflavonoid	30.012 ± 0.000	229sh, 275, 315sh
10	Flavone	31.478 ± 0.000	270sh, 294sh, 323
11	Benzoic acid derivative	30.290 ± 0.000	323
12	Quercetin-3-*O*-glycoside	30.660 ± 0.195	256, 266sh, 353
13	Unidentified	30.794 ± 0.313	229, 276, 308
14	Unidentified	30.646 ± 0.088	225sh, 275, 310sh
15	Benzoic acid derivative	31.229 ± 0.000	243
16	Quercetin-3-*O*-glycoside	31.637 ± 0.105	250sh, 267, 338
17	Flavonol	31.322 ± 0.196	222sh, 253, 288sh, 360
18	Quercetin-3-*O*-glycoside	31.479 ± 0.162	255, 266sh, 354
19	Benzoic acid derivative	31.655 ± 0.015	248
20	Quercetin-3-*O*-glycoside	32.278 ± 0.052	268, 336
21	Unidentified	32.410 ± 0.037	230sh, 276, 329
22	Dihydroflavonoid	32.123 ± 0.101	279, 320sh
23	Dihydroflavonoid	33.781 ± 0.000	239, 312sh
24	Flavone	33.928 ± 0.000	269sh, 303sh, 324
25	Quercetin-3-*O*-glycoside	34,035 ± 0.070	255, 269sh, 357
26	Kaempferol-3-*O*-glycoside	34.001 ± 0.000	269, 333
27	Flavone	34.297 ± 0.371	240, 318
28	Quercetin-3-*O*-glycoside	34.771 ± 0.081	253sh, 262, 346
29	Quercetin-3-*O*-glycoside	34.822 ± 0.000	257, 267sh, 296sh, 357
30	Unidentified	34.666 ± 0.270	220sh, 248
31	Herbacetin-3-*O*-glycoside	35.467 ± 0.278	266, 294sh, 318sh, 344
32	Flavone	35.189 ± 0.000	228, 276, 306
33	Flavone	35.774 ± 0.000	225sh, 251, 267, 326
34	Quercetin-3-*O*-glycoside	36.238 ± 0.066	254, 266, 344
35	Kaempferol-3-*O*-glycoside	36.670 ± 0.000	265, 345
36	Quercetin-3-*O*-glycoside	37.206 ± 0.140	254, 269sh, 351
37	Unidentified	37.398 ± 0.078	233sh, 268sh, 316
38	Benzoic acid derivative	37.565 ± 0.000	244
39	Cinnamic acid derivative	38.269 ± 0.000	239sh, 296sh, 323
40	Dihydroflavonoid	38.172 ± 0.396	230sh, 289, 334sh
41	Quercetin-3-*O*-glycoside	39.340 ± 0.000	226sh, 251sh, 269sh, 357
42	Quercetin-3-*O*-glycoside	39.180 ± 0.000	223sh, 255, 267sh, 298sh, 354
43	Flavone	40.264 ± 0.074	318
44	Benzoic acid derivative	40.875 ± 0.132	246
45	Quercetin-3-*O*-glycoside	40.772 ± 0.475	223sh, 252, 268sh, 348
46	Cinnamic acid derivative	42.566 ± 0.000	284sh, 314
47	Benzoic acid derivative	42.530 ± 0.037	240sh, 310
48	Cinnamic acid derivative	42.618 ± 0.052	277, 313

Table 2. Continuation.

Compound	Type of compound	Retention Time (min)	λ_{max} · (nm)
49	Quercetin-3-*O*-glycoside	42.923 ± 0.005	225sh, 255, 265, 300sh, 354
50	Quercetin-3-*O*-glycoside	42.641 ± 0.282	256, 266sh, 294sh, 355
51	Unidentified	43.271 ± 0.191	225sh, 268, 300
52	Cinnamic acid derivative	43.455 ± 0.238	235sh, 286, 315
53	Cinnamic acid derivative	44.811 ± 0.059	239, 311
54	Unidentified	44.784 ± 0.113	228sh, 285, 327sh
55	Benzoic acid derivative	45.076 ± 0.156	243, 288
56	Quercetin-3-*O*-glycoside	45.368 ± 0.000	227sh, 254, 270sh, 299sh, 372
57	Unidentified	45.764 ± 0.000	243
58	Quercetine-3-*O*-glycoside	46.036 ± 0.000	254, 262sh, 298sh, 357
59	Unidentified	46.350 ± 0.000	232sh, 281, 323sh
60	Dihydroflavonoid	47.758 ± 0.000	215sh, 227sh, 283, 317
61	Dihydroflavonoid	48.491 ± 0.206	233, 282
62	Cinnamic acid derivative	47.298 ± 0.000	219sh, 231sh, 278sh, 307
63	Dihydroflavonoid	47.858 ± 0.485	227sh, 289, 324
64	Cinnamic acid derivative	49.738 ± 0.000	305sh, 282sh, 314
65	Cinnamic acid derivative	49.269 ± 0.235	217sh, 233, 276, 316
66	Unidentified	50.325 ± 0.000	235sh, 275, 316
67	Dihydroflavonoid	50.903 ± 0.146	229sh, 283,
68	Kaempferol	52.940 ± 0.000	248sh, 266, 296sh, 320sh, 366
69	Isorhamnetin derivative	52.173 ± 0.059	228sh, 256, 270sh, 300sh, 329sh, 371
70	Dihydroflavonoid	52.794 ± 0.100	215, 229sh, 277, 313
71	Dihydroflavonoid	53.009 ± 0.000	213sh, 226sh, 292
72	Unidentified	53.977 ± 0.000	225sh, 260sh, 288, 336sh
73	Dihydroflavonoid	54.160 ± 0.000	228sh, 288, 331sh
74	Dihydroflavonoid	53.974 ± 0.465	228sh, 292
75	Dihydroflavonoid	54.916 ± 0.220	229sh, 292
76	Dihydroflavonoid	56.338 ± 0.000	281
77	Dihydroflavonoid	56.118 ± 0.000	225sh, 281
78	Dihydroflavonoid	58.870 ± 0.128	216sh, 227sh, 293, 334sh
79	Dihydroflavonoid	58.870 ± 0.128	227sh, 282
80	Dihydroflavonoid	60.602 ± 0.111	223sh, 294, 342sh
81	Dihydroflavonoid	62.499 ± 0.046	215sh, 230sh, 298, 237sh

synthesize and accumulate a relatively high number of flavonoid glycosides. The screening of flavonoid aglycones in those species would deserve further investigation for exploring their taxonomic significance, as well as for the role that those compounds play in protecting the plant tissues from damaging UV-B (280-315 nm) radiation (Williams and Harborne, 1994), and for the pharmacological properties those compounds show (Wollenweber, 1994). Quercetin-*O*-glycoside derivatives were the most abundant (18 compounds) among the flavonols. Derivatives of herbacetin (compound **31**), and isorhamnetin derivative (compound **69**) were other flavonols also found.

A complex flavanone (5,7dihydroxi-6,5'-di-metoxi-3', 4'-metilenedioxiflavanone) present in somatic tissues (Parmar *et al*., 1992), and of two flavonol glycosides (kaempferol-3-*O*-glucoside and kaempferol-3-*O*-rutinoside) from the flowers of *A. americana* (Subramanian and Nair, 1970) have been reported. Also, three complex isoflavanoids have been identified in the rhizomes of *A. barbadensis* (Tinto *et al*., 2005). Twenty three flavonoids were recently reported by Almaraz-Abarca *et al*. (2009) for adult foliar tissues of *A. durangensis*, all flavonol glycosides, most of them being derivatives of kaempferol. Those last authors also reported three kaempferol glycosides, as unique components of the phenolic profile for *Agave asperrima*. Flavonols, one of the three classes of flavonoids previously reported in species of *Agave* (flavanones, flavonols and isoflavonoids) were also found in the present study for *A. victoriae-reginae* s.l., *A. lechuguilla*, and *A. striata*, and contrary to those former reports, phenolic acids derivatives, flavones, dihydroflavo-

Table 3. Phenol compounds detected by HPLC/DAD in the foliar tissue from the studied populations of *A. victoriae-reginae* s.l., *A. lechuguilla*, and *A. striata*.
+ = present; - = absent; +/- = present or absent

Compound	*A. victoriae-reginae* s.l.								*A. lechuguilla*	*A. striata*
	Lerdo	Bustamante	Sta. Cata	Ramos Arizpe	Arteaga	Mina	Viesca	El Mezquital		
1	-	+/-	+	+	+	-	-	+/-	+/-	-
2	-	-	-	-	-	-	-	-	+/-	-
3	-	-	+/-	-	-	-	-	-	-	-
4	-	-	-	-	-	-	-	-	+/-	+/-
5	-	-	-	-	-	-	-	+/-	-	-
6	-	-	-	-	-	-	-	-	-	+/-
7	+/-	+/-	+/-	-	-	-	-	+/-	+/-	-
8	+/-	+/-	+/-	-	+/-	-	-	-	-	-
9	-	+/-	+/-	-	-	-	-	-	+/-	-
10	-	-	-	-	+/-	-	-	-	-	-
11	+/-	+/-	+/-	+/-	-	-	-	-	-	-
12	-	-	-	-	-	-	-	-	-	+/-
13	-	-	+/-	-	-	-	-	-	-	-
14	-	-	-	-	-	-	-	-	+/-	-
15	-	+/-	-	-	-	+	-	-	-	+/-
16	-	-	-	-	-	-	-	+/-	-	-
17	-	-	-	-	-	-	-	-	-	+/-
18	-	-	-	-	-	-	-	-	-	+/-
19	+/-	+/-	-	-	-	-	-	+/-	-	-
20	+/-	+/-	+/-	-	+/-	-	-	+/-	+/-	-
21	-	-	+/-	-	-	-	-	-	+/-	-
22	-	-	-	-	-	+	-	-	-	-
23	+/-	+/-	-	-	-	-	-	-	-	-
24	-	+/-	+/-	-	+/-	+/-	-	+	-	-
25	-	+/-	+/-	-	+/-	+	-	-	+/-	-
26	-	-	-	-	-	-	-	-	-	+/-
27	-	-	+/-	-	-	-	-	-	-	-
28	-	-	-	-	-	-	-	-	-	+/-
29	+/-	+/-	-	+/-	+/-	-	-	-	-	-
30	+	+/-	-	-	-	-	-	-	-	-
31	-	-	-	-	-	-	-	-	-	+/-
32	-	-	+/-	+/-	-	+	-	-	-	-
33	-	-	-	-	-	-	-	-	+/-	+/-
34	-	-	-	-	-	-	-	-	-	+
35	-	-	-	-	+/-	-	-	-	+/-	-
36	+/-	+/-	-	-	+/-	+/-	-	-	+/-	-
37	-	-	-	-	-	-	-	+/-	-	-
38	-	-	+/-	-	-	-	+	+/-	-	-
39	-	-	+/-	-	-	+	+	+/-	+/-	-
40	-	-	-	-	-	-	-	-	-	+/-
41	+/-	-	-	-	-	-	-	-	-	-
42	-	-	-	-	-	-	-	-	-	+
43	+/-	+	+	+	+	+	-	+	-	-
44	-	-	-	-	-	-	-	-	-	+/-
45	-	-	-	-	-	-	-	-	-	+
46	+/-	+/-	+/-	+/-	+/-	+/-	-	-	+/-	-
47	-	+/-	-	-	+/-	-	-	-	-	-

Table 3. Continuation.

Compound	*A. victoriae-reginae* s.l.								*A. lechuguilla*	*A. striata*
	Lerdo	Bustamante	Sta. Cata	Ramos Arizpe	Arteaga	Mina	Viesca	El Mezquital		
48	+/-	+/-	+/-	+/-	+/-	+/-	-	-	-	-
49	-	-	-	-	-	-	-	-	-	+/-
50	-	-	-	-	-	-	-	-	-	+
51	-	+/-	-	+/-	+/-	-	-	-	-	-
52	-	-	-	-	-	-	-	-	+/-	+/-
53	-	-	+/-	+/-	-	-	-	-	-	-
54	-	-	-	-	-	-	-	-	+/-	-
55	+/-	-	-	-	-	-	-	-	-	-
56	-	+/-	-	-	-	-	-	-	-	-
57	-	-	+/-	-	-	-	-	-	-	-
58	-	-	-	-	-	-	-	-	-	+/-
59	+/-	-	+/-	+/-	-	-	+	+/-	+/-	-
60	+/-	+/-	-	-	+	-	-	-	-	-
61	-	-	-	-	-	-	-	-	-	+/-
62	-	-	+/-	-	-	-	-	-	-	-
63	-	-	-	-	-	-	-	-	-	+/-
64	-	-	+/-	+/-	-	+/-	-	-	-	-
65	+/-	+/-	-	+/-	+/-	-	+	-	+/-	-
66	+/-	-	+/-	+/-	-	-	-	+/-	-	-
67	-	+/-	+/-	-	+/-	-	+	+/-	-	-
68	-	+/-	-	-	-	-	-	-	-	-
69	+/-	+/-	-	-	-	-	+	-	-	-
70	-	-	-	-	-	-	-	-	-	+
71	-	-	-	-	-	-	-	-	+/-	-
72	+/-	-	+/-	-	+/-	+	-	+/-	-	-
73	-	-	-	-	-	-	-	-	+	+
74	+	+/-	-	-	-	-	+/-	-	+/-	-
75	+/-	+/-	-	-	+	-	+	-	-	-
76	-	+/-	-	+/-	+/-	-	-	+/-	-	-
77	-	-	-	-	-	-	-	+/-	-	-
78	-	-	-	-	-	-	-	-	-	+
79	-	-	-	-	-	-	-	-	+/-	-
80	-	-	-	-	-	-	-	-	+/-	-
81	-	-	-	-	-	-	-	-	+/-	-

noids, and a higher diversity of flavonols, including derivatives of herbacetin, and ishoramnetin were detected (Figure 1, Table 2). Phenolic acids provide the precursors for lignin, tannins, and flavonoid biosynthesis (Hrazdina, 1992; Heller and Forkmann, 1994). In species of *Agave*, which do not accumulate relevant amounts of foliar lignin neither tannins (Wall *et al*., 1957; Seigler, 2002), phenolic acids could have several other functions, like protection against herbivores and pathogens, action as cementing material joining phenolic polymers to polysaccharides of cell walls (Wallace and Fry, 1994), participation in the regulation of cell growth and division (Binns *et al*., 1987), inhibition of ion intake by a generalized increase in membrane permeability to inorganic ions as a mode of action of allelopathic compounds (Glass and Dunlop, 1974), protection to thermal stress (Dixon and Paiva, 1995), and function as antioxidants (Rice-Evans *et al*., 1997; Almaraz-Abarca *et al*., 2007), which could give survival advantages to *A. victoriae-reginae* s.l., *A. lechuguilla*, and *A. striata*, that synthesize those phenols.

Taxonomic significance. Fifty one phenols were found by HPLC/DAD in the foliar tissue of *Agave victoriae-reginae* s.l. (considering the eight populations). From those, 45 were found in *A. victoriae-reginae* s. str., excluding the populations of Ramos Arizpe and Arteaga, which correspond to *A. nickelsiae*, and the population of El Mezquital, which corres-

Table 4. Concentration (mg g^{-1} dried tissue) of selected phenolic compounds present in the foliar tissues of *A. victoriae-reginae* s.l., *A. lechuguilla*, and *A. striata*. The values represent the mean and standard deviation for the corresponding number of individuals analyzed. Values in parenthesis correspond to samples collected in October. -: absent

Compound	*A. victoriae-reginae* s.l.								*A. lechuguilla*	*A. striata*
	Lerdo	Bustamante	Sta. Catarina	Ramos Arizpe	Arteaga	Mina	Viesca	El Mezquital		
4	-	-	-	-	-	-	-	-	20.6 ± 14.5	21.0 ± 8.1
5	-	-	-	-	-	-	-	3.95 ± 1.2 (12.58 ± 5.0)	-	-
8	47.8 ± 17.9	16.0 ± 14.7	29.3 ± 8.8	-	24.5 ± 9.8	-	-		-	-
11	30.64 ± 4.2	56.5±2.6	21.2 ± 5.8	23.8 ± 6.5	-	-	-	-	-	-
29	38.1 ± 1.8	27.5 ± 15.7	-	30.42 ± 11.9	35.5 ± 13.2	-	-	-	-	-
33	-	-	-	-	-	-	-	-	33.9 ± 5.8	7.7 ± 4.2
37	-	-	-	-	-	-	-	15.2 ± 12.7 (17.38 ± 8.1)		
48	49.3 ± 7.8	26.6 ± 19.7	51.5 ± 8.1	39.9 ± 5.3	56.2 ± 16.9	60.6 ± 29.4	-	-	-	-

ponds to *A. pintilla*, according to González-Elizondo *et al.* (2011). For *A. striata* were detected 24, and also 24 (most of them different) in *A. lechuguilla* (Table 3). This distribution of phenols indicates that the group most diverse, regarding these secondary metabolites, is that of *A. victoriae-reginae*. That chemical richness is the highest reported yet for any species of *Agave* if compared with the reports of Subramanian and Nair (1970), and Parmar *et al.* (1992) for *A. americana*; Tinto *et al.* (2005) for *A. barbadensis*; Chen *et al.* (2009) for *A. sisalana*; and Almaraz-Abarca *et al.* (2009) for *A. asperrima*, *A. durangensis*, *A. shrevei* subsp. *shrevei*, *A. shrevei* subsp. *matapensis*, and *A. wocomahi*. Nineteen (compounds **6**, **12**, **17**, **18**, **26**, **28**, **31**, **34**, **40**, **42**, **44**, **45**, **49**, **50**, **58**, **61**, **63**, **70**, and **78**) among the 24 phenols forming the profile of *A. striata* were unique to this species, and six of them (compounds **34**, **42**, **45**, **50**, **70**, and **78**) were present in every analyzed individual of this taxon; while seven (compounds **2**, **14**, **54**, **71**, **79**, **80**, and **81**) among the 24 phenols detected for *A. lechuguilla* were only present in this species (Table 2). A higher number, thirty seven (compounds **3**, **5**, **8**, **10**, **11**, **13**, **16**, **19**, **22**, **23**, **24**, **27**, **29**, **30**, **32**, **37**, **38**, **41**, **43**, **47**, **48**, **51**, **53**, **55**, **56**, **57**, **60**, **62**, **64**, **66**, **67**, **68**, **69**, **72**, **75**, **76**, and **77**) among 51 phenols, was detected only in *A. victoriae-reginae* s.l. (Table 2). The predominance of phenolic acids in *A. victoriae-reginae* s.l. and of flavonols, majorly quercetin-*O*-glycosides, in *A. striata*, can be observed in Figure 3. *Agave striata* was collected at the same location than one of the populations of *A. victoriae-reginae*, at Lerdo, Durango (Table 1), where they are sympatric, exposed to the same environmental conditions. This allows to conclude that the evident differences in their foliar phenol profiles are the result of genetic differences that command species-specific sequential order in the biosynthesis pathway, as Heller and Forkmann (1994) pointed out, of which many of individual steps of the flavonoid modification have shown to be controlled by single genes (Forkmann, 1994). Our results indicate a species-specific tendency of phenol profiles for those taxa, as has been found for other groups of plants (Bate-Smith *et al.*, 1975; Campos, 1997; Almaraz-Abarca *et al.*, 2006).

The remarkable differences in the phenol profiles between *Agave victoriae-reginae* s.l. and *A. striata* correspond to the fact that the former belongs to a different series (Marginatae) than *A. striata*, which belongs to the series Striatae (Gentry, 1982). And although *Agave lechuguilla* is closer to *A. victoriae-reginae* s.l. (both belonging to the series Marginatae), the differences in the phenol profiles can also be observed between them (Table 3). In both, *A. lechuguilla* and *A. victoriae-reginae* s.l., flavonols are not predominant, but in *A. lechuguilla* a codominance of flavonols and dihydroflavonoids was detected, unlike *A. victoriae-reginae* s.l., in which phenolic acids are abundant (Figure 2). *Agave lechuguilla* grows also in Lerdo, Durango, under the same environmental conditions that *A. victoriae-reginae* and *A. striata*, so that differences in the phenolic profiles can be also assumed as specific expression patterns.

Although flavonoids are the major chemotaxonomic markers (Markham, 1982; Harborne and Turner, 1984), phenolic acids also possess a certain value as chemical markers (Van Sumere, 1989), and may be ubiquitous and accumulated at significant levels in plant tissues (Tamagnone *et al.*, 1998). Pollen of some plant species may be particularly rich in phenolic acids (Campos, 1997; Almaraz-Abarca *et al.*, 2004; Almaraz-Abarca *et al.*, 2007) but these may be completely missing in the pollen of others, as in *A. durangensis* (Almaraz-Abarca *et al.*, 2009). To our knowledge, the ubiquitous richness and diversity of benzoic acid and cinnamic acid derivatives in the foliar tissue of *Agave*, particularly of *A. victoriae-reginae* s.l. and *A. lechuguilla*, are reported for the first time.

The presence of phenolic acids in the species here studied, all of them belonging to *Agave* subgenus *Littaea*, contrasts with their absence in *A. americana* (Parmar *et al*., 1992), *A. asperrima*, *A. durangensis*, *A. shrevei*, *A. wocomahi*, (Almaraz-Abarca *et al*., 2009), and *A. sisalana* (Chen *et al*., 2009), which belong to *Agave* subgenus *Agave*. If those tendencies are found in more species of both subgenera, it could be suggested that the relative abundance of phenolic acids may have some taxonomic relevance at the subgeneric level in *Agave*. The trend of a taxon to synthesize preferentially one phenol type rather than other one could be an indicator of evolutionary tendencies within Agavaceae, as has been established for Asteraceae by Emerenciano *et al*. (2001), among other many dicotyledonous, and for monocotyledonous other than Agavaceae, e.g., for Juncaceae (Williams and Harborne, 1975), Liliaceae (Williams, 1975), Fluviales (Harborne and Williams, 1976a), Gramineae (Harborne and Williams, 1976b), and Orchidaceae (Williams, 1979). To establish the evolutionary connotation of the flavonoid accumulation in this group is necessary to analyze more taxa of monocots, since some differences have been detected with respect to the tendencies found in dicotyledons, as has been pointed out by Harborne (1977).

The species of *Agave* here analyzed showed some chemical characters reported by Salatino *et al*. (2000) as primitive in Ericaulaceae (a monocotyledonous family), such as complex patterns of flavonoids (formed by several different types of phenols). Some chemical features, such as the accumulation of methylated flavonoids, considered as evolved for *Pinus* (Kaundun and Lebreton, 2010), were found in this study (compound **69**, an isorhamnetin derivative) for plants from Lerdo, Bustamante, and Viesca, all belonging to *A. victoriae-reginae* s.str. To establish the evolutionary tendency of the accumulation of phenolic compounds in Agavaceae, and particularly in *Agave*, more detailed analysis on a broader number of species are needed.

Martínez-Palacios *et al*. (1999) mentioned for *Agave victoriae-reginae* high levels of genetic variability within populations as well as high levels of interpopulation differentiation. This variability is also observed in the foliar phenol profiles exhibited by the different populations here analyzed (Table 3, Figures 3, 4). Six among the eight populations of *A. victoriae-reginae* s.l. (Santa Catarina, El Mezquital, Arteaga, Mina, Lerdo, and Bustamante) displayed one to six unique compounds, not found in any other. Thus, compound **22** was found only in the population of Mina; compounds **41** and **55** were detected in Lerdo; compounds **56** and **68** in Bustamante; compounds **10** and **35** in Arteaga; compounds **5**, **16**, **37**, and **77** in El Mezquital; and compounds **3**, **13**, **21**, **27**, **57**, and **62** in Santa Catarina.

This distribution of phenol profiles corresponds partially to the separation of taxa based on morphological traits. The population of El Mezquital, the most isolated geographically, which corresponds to a different species, namely *A. pintilla* (González-Elizondo *et al*., 2011), is distinguished by possessing four unique compounds. But phenol profiles did not allow to distinguish between *Agave victoriae-reginae* s. str. and *A. nickelsiae*, as found by Martínez-Palacios *et al*. (1999) using allozymes. In the Figure 4, the eight populations of *Agave victoriae-reginae* s.l. formed four groups: (A) Viesca; (B) Arteaga with some individuals from Bustamante, and Lerdo; (C) Mina, Santa Catarina, Ramos Arizpe, and some individuals from Bustamante; and (D) El Mezquital. The separation of all the individuals of El Mezquital from the rest is consistent with the proposal, based on morphological attributes, of non conspecificity for this population, and of considering to *A. victoriae-reginae* as a complex instead of a single species (González-Elizondo *et al*., 2011). However, some inconsistencies were found among our chemical results and the ones reported by González-Elizondo *et al*. (2011) based on morphological traits and by Martínez-Palacios *et al*. (1999) based on allozymes. Excluding the population of El Mezquital, which was not analyzed by Martínez-Palacios *et al*. (1999), the three other groups (Figure 4) did not clearly correspond to the groups proposed by either González-Elizondo *et al*. (2011) or Martínez-Palacios *et al*. (1999), except for group (C) (Figure 4), which roughly corresponds to the eastern populations found by Martínez-Palacios *et al*. (1999). The incongruence between our results and those reported by those authors are comparable to those reported between morphological and molecular features for other species of *Agave* (Alfaro *et al*., 2007). Incongruence between different kinds of markers has been explained by Hörandl (2010) as a result of an asynchronous marker evolution.

The foliar tissues of *Agave victoriae-reginae* s.l., *A. lechuguilla*, and *A. striata* have complex profiles of phenols, which are rich in number and classes of those compounds, including phenolic acids, flavonols, flavones, and dihydroflavonoids. The three taxa can be distinguished by the different qualitative and quantitative phenol patterns. The presence of unique phenols in the different populations of *A. victoriae-reginae* s.l. suggests the recognition of four chemotypes in that variable group. Phenol composition supports the proposal of considering the population of El Mezquital as an independent species (*A. pintilla*).

Quantitative variations. Variations in the amount accumulated of some compounds could be detected among the different populations of *Agave victoriae-reginae* s.l., *A. lechuguilla*, and *A. striata* (Table 4). Concerning the levels of the compounds **8**, **11**, **29**, and **48**, the populations of Lerdo, Bustamante, Santa Catarina, Ramos Arizpe, Arteaga, and Mina (all sampled in May) showed different levels of them, suggesting that those variations depend on the geographical origin. The difference observed in compound **33**, a flavone (Table 4), common to *A. lechuguilla* and *A. striata*, collected at the same season and the same location, suggests that im-

portant variation can be found between species synthesizing the same compound. Considerable variation in concentrations were observed in the compounds **5** and **37** (unidentified), both in the population of El Mezquital (*A. pintilla*) between individuals sampled in May-June and those sampled in October (Table 4), with an increased concentration in October with respect to May-June, suggesting fluctuations correlated to seasonal changes in adult plants of *Agave*. This increase in concentration could be associated to a response to the increase of herbivores and pathogenic fungi and bacteria attack due to an increase of the precipitation, since phenols are known to be active against those organisms (Echeverri *et al*., 1991; Hadacek, 2002).

Due to their stability, qualitative phenolic profiles have more taxonomic significance than quantitative patterns (Harborne and Turner, 1984); however, further investigation needs to be performed to determine the fluctuation patterns associated to different environmental conditions and also to developmental stages of the accumulated phenolics in *Agave*, not only with taxonomic aims but also in order to extend our knowledge on the role and synthesis regulation of those compounds in the genus.

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