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Profiling of Phenolic Compounds of Somatic and Reproductive Tissues of Agave Durangensis Gentry (Agavaceae)

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Abstract: Problem statement: In Durango, Mexico, mescal is elaborated from wild plants of *Agave durangensis*. This species shows a high morphological variability within and among populations, what makes its taxonomic delimitation a hard task. **Approach:** In this study the pollen and foliar phenolic compositions of *Agave durangensis* were analyzed by HPLC/DAD with the aim of determining the significance of phenol profiles to delimit this taxon. **Results:** The foliar phenol compositions were evaluated within and among two populations and between juvenile and adult plants. *Agave asperrima* Jacobi, *Dasylirion* sp. and juvenile samples of *A. shrevei* Gentry subsp. *shrevei*, *A. shrevei* Gentry subsp. *matapensis* Gentry and *A. wocomahi* Gentry, were also analyzed to stand comparisons with. The results from this study indicated that pollen and foliar tissues of *Agave durangneisis* were rich in kaempferol glycoside derivatives (13 and 23 different compounds can be present, respectively). Principal coordinates analysis (PCO), based on foliar profiles of adults, indicated the presence of several chemotypes within the Type locality of *Agave durangensis* and revealed chemical differences between the both analyzed populations. **Conclusion/Recommendations:** Chemical and morphological differences and biogeographical evidence suggest the recognition of two different taxonomic entities in this morphological variable group.

Key words: Pollen flavonoids, foliar flavonoid profiles, *Agave* phenolic variability

INTRODUCTION

Agave is the biggest genus of the family *Agavaceae*, with around 166 species, from which 125 grow in Mexico[1,2]. Relevant ethnobotanic relationships have been established between the elements of this genus and the ancient and present cultures of Mexico[3,4]. Several authors have described the use of *Agave* as source of fibers, food and beverages[5]. In addition, *Agave* is used as natural fences to avoid the soil erosion and as cattle food[6].

At the present, the relevance of *Agave* has increased meanly because of the increased demand of alcoholic beverages like tequila and mescal[7] and the research on potential sources of prebiotics[8]. In all the cases, the authentification of the species of *Agave* to be used is an important requirement in the quality control of the manufacturing processes. In Mexico, with exception of *Agave tequilana* Weber var. azul, species like *Agave durangensis*, which support local industries of alcoholic beverages and which with just begin an industrialization process, techniques are needed to guarantee the botanical origin of plants, according to the respective origin denomination statement.

Agave durangensis Gentry is one of the 24 species of genus *Agave* occurring in Durango, Mexico[9]. This species belongs to group *Ditepalae* of subfamily Agavoideae, proposed by Gentry[3] and can be found from Southern Durango to Northern Zacatecas[3,10].

In two localities of Durango ("Sierra of Registro", the type locality and "Mezquital"), populations of *Agave*, traditionally called "agave mezcalero" or "agave cenizo", presumably *A. durangensis*, maintains a thriving mescal industry. Actually, studies have not been done to confirm that in all the cases the raw material to elaborate mescal is composed of that species[10]. In Durango, the manufacturing of mescal is mainly based on the gathering of agaves from their natural populations[7]. Recently, producers have been interested in the establishment of plantations of

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A. durangensis and then, typification methods are needed to guarantee the botanical origin of plants, since genus *Agave* is taxonomically difficult, owing to the high degree of phenotypic plasticity, the occurrence of several ploidy levels and the high capacity of hybridization[3,4]. Previous reports[3] and our own field observations in the Type locality ("Sierra of Registro", Durango, Mexico) indicate that *A. durangensis* is highly variable in size, color of leaf and size and form of teeth, in such a way that it has been suggested as a complex of species more than a single species by some authors[7]. Chemical characterization is an important technique, which with plant typification and identification can be made in a relative easy and fast manner, as it has been reported for many groups of plants[11,12].

In the literature there are many reports describing the significance of phenol profiles to discriminate among different related taxa[13-15]. The species-specific tendency of pollen[12,16,17] and somatic tissues phenol profiles[18,19] has been reported for many species of plants. In spite of the economic and ecologic importance and the taxonomic controversies prevailing in the delimitation of the different species of the genus *Agave* and in the other hand, in spite of the taxonomic relevance of phenol composition, the efforts focused in determining the taxonomic significance of the phenol profiles in this genus has been limited.

Agave is rich in saponins and it has been better analyzed by its saponin composition[20-23] than any other secondary metabolite. One of the few species of *Agave* analyzed by its phenol composition is *Agave americana*, in which complex flavonoids has been found in somatic tissues, like 5,7-dihydroxy-6,5'-dimethoxy-3',4'-methylenedioxyflavanona[24] and kaempferol-3-glycoside derivatives were detected in flowers[25]. In this study the phenol characterization of somatic and reproductive tissues of *Agave durangensis* was performed, using high pressure liquid chromatography/diode array detector (HPLC/DAD) profiles in order to establish the specific taxonomic significance of these markers and to detect intra and interpopulation variability. Moreover, to our knowledge, the phenolic composition of *Agave durangensis* and *A. asperrima* is reported for the first time.

MATERIALS AND METHODS

Plant material: Foliar tissue from 23 adult plants (no less than 22 leaves) of each two populations of *Agave durangensis*, were analyzes for their phenol composition by HPLC/DAD. With comparative aims, an equal number of individuals of *Agave asperrima* was collected and analyzed in the same manner. Mature flowers of four individuals from two different populations of *A. durangensis* were also collected for phenol composition analysis. Nineteen juvenile foliar samples of *A. durangensis* (plants of no more than eight leaves) were as well collected and analyzed for phenol composition. Reference samples were deposited at Herbarium CIIDIR. Juvenile foliar samples of *Agave shrevei* subsp. *shrevei* (28785), *A. shrevei* subsp. *matapensis* (AG-8922), *Agave wocomahi* (28891) and *Agave durangensis* (AG-5973), all donated by Dr. Abisai García-Mendoza, from the National Colection of Agavaceas, Botanic Garden, Biology Institut, UNAM and foliar samples of *Dasylirion* sp. (Nolinaceae) from four individuals growing at CIIDIR-IPN-Dgo., were analyzed for comparisons. The ecogeography profile for each sampling sites is shown in Table 1. Additionally, information about the morphological features was recorder for each sample.

Phenol extraction: Each sample was individually treated. Five grams of foliar dried grinded tissue were firstly extracted by maceration for 24 h, in 100 mL 60% methanol (v/v), in darkness and at room temperature.

Table 1: Collection sites for *Agave durangensis* and *Agave asperrima*

Sample	No. Ref.	Species	Location	Latitude N	LongitudeW	Altitude (m)	Soil	Associated vegetation	Date
1 and 2	531 and 533	*A. durangensis*	Temoaya, Mezquital, Durango	23°29' 04''	104°26' 31''	1780	Gravel	*Dasylirium* sp., *Lippia* sp., *Selaginella* sp., *Acacia* sp.	Jun 08
3 and 4	601 and 603	*A. durangensis*	Mezquital, Durango	23°37' 47'"	104°22' 08"	1855	Gravel	*Dasylirium* sp., *Bursera* sp., *Lippia* sp.	Jun 08
5-27	202-233	*A. durangensis*	Sierra El Registro, Durango	23°59' 38''	104°22.5' 13"	1928	Sandy	*Prosopis* sp. *Acacia* sp., *Dasylirium* sp.	Jun 08
28-50	500-533	*A. durangensis*	Temoaya, Mezquital, Durango	23°29' 04''	104°26' 31''	1780	Gravel	*Dasylirium* sp., *Lippia* sp., *Selaginela* sp., *Acacia* sp.	Jun 08
51-73	300-333	*A. asperrima*	Cuencamé, Durango	25°01' 0.5''	103°45' 51''	1442	Gravel	*Opuntia* sp., *Fouqueiria* sp., *Euphorbia antisyphilitica, Larrea tridentate*	Jun 08

The extracts were centrifuged (5000 rpm), for 10 min, at room temperature. The supernatants were separated. The pellets were reextracted in 100 mL 60% methanol (v/v) by maceration for 3 h. The extracts were centrifuged at the same conditions. The similar supernatants were brought together and formed the total extracts. Each total extract was concentrated under vacuum to dryness and then resolved in 5 mL methanol; aliquots were taken to be used in the HPLC/DAD analysis.

Samples of pollen collected directly from anthers were individually extracted according to Campos[11], with ethanol-water (50% v/v) and sonicated for 60 min. The resultant mixtures were centrifuged for 10 min and the supernatants used for HPLC/DAD analysis.

HPLC/DAD analysis: To determine the HPLC/DAD phenolic profiles, extracts (20 µL) were analyzed as previously described[16], on a Perkin Elmer HPLC system and Perkin Elmer Brownlee Analytical C18 column (4.6×250 mm, 5 µm), by an acidified acetonitrile-water gradient. Standard chromatograms were plotted at 260 and 340 nm. Spectral data for all peaks were accumulated in the range 220-400 nm using diode-array detection (Perkin Elmer Series 200). The structural identifications were made by direct comparison of retention time and UV spectra of standards and according to[26,27].

Data analysis: The phenol profile of each sample was made up of all compounds resolved in their respective HPLC/DAD chromatograms. Each compound was treated as a single chemical character. A binary matrix of presence-absence formed by all individual samples vs. all resolved compounds, for each adult and juvenile samples, were analyzed by principal coordinates analysis (PCO) from NTSyS 1.8[28].

RESULTS

Pollen phenol composition: The relation of phenolic compounds found in pollen of *Agave durangensis* is shown in Table 2. Retetion times and the chemical identification are included. Individual variability can be observed.

Foliar phenol composition: The resolved flavonoid compounds for all the 23 individuals of *Agave durangensis* from Sierra of Registro are presented in Table 3. The individual foliar phenolic profiles of each sample from the population of "Temoaya" are indicated in Table 4; while those corresponding to *A. asperrima* are shown in Table 5. In all these cases, the retention times and the chemical identification are included. As in pollen, variability among the individual profiles can be observed.

The foliar phenol profiles of each of the four analyzed individuals of *Dasylirion* sp. are showed in Table 6. Contrary to that observed for samples of *Agave*, individual variability was not observed.

Principal coordinates analysis: The discrimination of taxa based on the foliar phenol composition of adults, according to the PCO analysis, is showed in Fig. 1. The clear segregation of *Dasylirion* sp., *A. asperrima* and *A. durangensis* from "Temoaya" can be observed. However, three subgroups in the mayor group of *A. durangensis* from Sierra of Registro can be distinguished. The PCO analysis, based on the foliar phenol composition of juvenils, is showed in Fig. 2. The segregation of samples of *A. shrevei* subsp. *shrevei*, *A. shrevei* subsp. *matapensis* and *A. wocomahi* and the inclution of the sample of *A. durangensis* from the Botanic Garden, UNAM, in the mayor group formed by the most of the juvenile samples of *A. durangensis* from Sierra of Registro can be observed.

Table 2: Chromatographic data for pollen phenol compounds of *Agave durangensis*

Compound	Retention time (min)*	Chemical identification	531	533	601	603
P1	28.349±0.020	Kaempferol glycoside	+	+	+	+
P2	29.491±0.050	Kaempferol glycoside	+	+	-	-
P3	31.618±0.053	Kaempferol glycoside	-	-	+	+
P4	29.938±0.000	Kaempferol glycoside	+	-	-	-
P5	31.917±0.115	Kaempferol glycoside	-	-	+	+
P6	30.716±0.000	Kaempferol glycoside	+	-	-	-
P7	31.836±0.000	Kaempferol glycoside	-	+	-	-
P8	32.149±0.049	Kaempferol-3-O-[glucosyl(1-2)glucoside]	-	-	+	+
P9	32.537±0.000	Myricetin-3-O-rhamnoside	-	-	+	+
P10	31.985±0.060	7-O-methylkaempferol-3-O-[rhamnosyl(1-2)glucoside]	+	+	-	-
P11	33.118±0.219	Kaempferol glycoside	-	-	+	+
P12	35.198±0.033	Kaempferol-3-O-glucoside	+	+	-	-
P13	37.5305±0.303	Kaempferol glycoside	+	+	-	-
		Total	7	6	6	6

+: Present; -: Absent; *: Mean and standard deviation

Table 3: Individual distribution of foliar flavonoid compounds of *Agave durangensis* from "Sierra of Registro"

Comp.	Retention time (min)	Chem. Ident.	200	201	202	203	204	206	209	210	211	213	214	216	218	219	221	224	225	228	229	230	231	232	233
F1	18.227± 0.093	KG	0	0	0	0	0	0	0	0	0	1	0	0	1	0	0	0	0	0	0	0	0	0	0
F2	18.865±0.00	KG	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0
F3	19.598±0.035	KG	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	1	0	0
F4	22.489±0.042	KG	0	0	0	0	0	0	0	0	0	1	1	0	1	1	0	1	0	1	0	0	0	0	0
F5	23.326±0.089	KG	0	0	0	1	1	0	1	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0
F6	24.438±0.00	KG	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0
F7	25.142±0.00	QG	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0
F8	27.067±0.00	QG	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
F9	27.710±0.060	KG	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	1	1	0	0	0	0	0
F10	28.313±0.014	KG	0	0	1	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	1	1	0	0	1
F11	27.623±0.051	K3,7OG	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	0	0
F12	29.955±0.104	KG	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
F13	29.969±0.090	KAcG	0	0	0	1	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0
F14	31.610±0.056	KG	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
F15	31.434±0.131	KG	1	1	1	1	1	1	1	1	1	0	1	1	0	1	1	1	1	1	1	1	1	0	1
F16	32.506±0.126	KG	0	0	0	0	1	0	0	0	1	0	1	1	1	0	1	1	1	0	1	1	0	1	1
F17	33.238±0.095	KG	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
F18	33.609±0.051	KG	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	1
F19	34.937±0.086	KRhG	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	1	0	0	0
F20	34.720±0.00	QA	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0
F21	35.603±0.154	KG	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
F22	35.445±0.046	KG	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0
F23	37.148±0.169	KG	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
F24	38.191±0.030	KRh	1	1	1	1	1	1	1	0	1	0	1	0	0	1	1	1	1	1	1	1	0	1	1
F25	37.198±0.096	KG	0	0	0	0	0	0	0	0	0	0	1	1	0	1	0	0	0	0	0	0	0	0	0
F26	38.577± 0.236	KG	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	1	0
F27	38.874±0.00	KG	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
F28	40.004±0.120	KG	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
F29	40.074±0.00	KG	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0
F30	40.924±0.00	KG	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0
F31	48.528±0.057	KG	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
F32	50.385±0.161	KG	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

*: Mean and standard deviation; KG: Kaempferol Glycoside; QG: Quercetin Glycoside; K3,7OG: Kaempferol-3,7-O-diglucoside; KAcG: Kaempferol-3-O-[6-acetylglucoside]-7-O-glucoside; KRhG: Kaempferol-3-O-[rhamnosyl(1-6) glucoside]; QA: Quercetin-3-O-arabinoside; KRh: Kaempferol-3-O-rhamnoside; 1: Present; 0: Absent

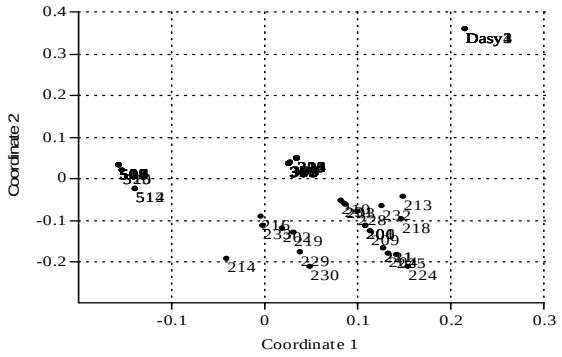

Fig. 1: Results of Principal Coordinates Analysis comparing foliar flavonoid profiles for samples of *Agave durangensis* from Sierra of Registro (200-233), *A. durangensis* from "Temoaya" (500-522), *A. asperrima* (300-326) and *Dasylirion* sp. (Dasy1-Dasy4)

DISCUSSION

Taxonomic implications of pollen phenol composition: A total of 13 different compounds were detected by HPLC/DAD (Table 2). Flavonoids were the only class of phenolics found in the pollen of *Agave durangensis*. The analysis revealed 12 kaempferol glycosides and one myricetin glycoside. Derivatives of both quercetin and kaempferol are the most abundant phenols in pollen[11,12,17,29]. They along with myricetin are involved in such as important functions as the pollen tube germination and growth in several species of plants[30,31]; however, in spite of quercetin glycoside derivatives are almost ubiquitous, it was significantly absent from *Agave durangensis* pollen. The role of the abundant kaempferol glycosides in pollen of *Agave durangensis* is left for determining.

The pollen phenol profile of the two individuals of *Agave durangensis* coming from the population of "Mezquital" were identical between them (six flavonols and the presence of one myricetin glycoside) but some

different of those of the two individuals from "Temoaya", which were very similar between them but a little variability was detected (Table 2). These two localities are separated one from each other by 15 Km and similar morphological traits between both populations were observed.

Variability in pollen phenol profiles has been showed for other plants, including plants sharing the habitat of *A. durangensis* in the semiarid zones of Durango, like some species of Cactaceae[12].

The importance of pollen phenol profiles as specific taxonomic markers has been reported[16,17]. These results suggest that, although only two individuals from each population were analyzed (inflorescences of *Agave durangensis* are difficult to find due to it is a monocarpic species and due to when plants are ready to flower, the inflorescences stems are cut to prepare the plants for mescal manufacturing), this kind of profiles could be even used to discriminate among populations. This would be relevant because of the taxonomic controversy in the specific delimitation of *A. durangensis*, which is, in turn, a consequence of the high morphological variability found in this taxon[3,10] and of the little effort dedicated to study this group. However, to propose these profiles as a specific and infraspecific marker, it is necessary to carry out more population studies, with a higher number of individuals, throughout all the geographical distribution.

Taxonomic implications of foliar phenol composition: The flavonoids of the *Agave* family, the Agavaceae, have not been well investigated; just a few species have been analyzed[32], so that, this survey, basically of two species of *Agave* (*A. durangensis* and *A. asperrima)*, is useful in indicating the variation that may be encountered in these plants.

A total of 32 compounds were resolved in the foliar samples of adult plants by HPLC/DAD (Table 3). The analysis revealed 23 flavonol glycosides present in the foliar profiles of *Agave durangensis*. Twenty of those were kaempferol glycoside derivatives. Three different quercetin glycoside derivatives were found in samples 203, 225 and 228. More phenol compounds could be present but at a very low concentration in such a way that their identification was not possible.

A high variability was found in the individual phenol profiles of the population from Sierra of Registro, which is the Type locality of *Agave durangensis*. Our own field observations revealed also a high morphological variability concerning the leaves form and length, adult plant height, number of teeth in 10 cm and terminal spine length, this variability agree to that found in the phenol composition. The profiles varied from three compounds in four individuals (200, 201,206 and 232) to nine in the individual 230 (Table 3).

The PCO analysis based on the foliar phenol composition of the individuals from Sierra of Registro revel the presence of three major groups; these could represent three chemotypes (Fig. 1). The morphological and chemical variations could reflect a present evolution process in this taxon, which has been put under an intense environmental pressure due to the overexploitation and deforestation of the area several decades ago.

As in the case of pollen, the dominance of kaempferol derivatives was clear in the foliar phenol composition of *A. durangensis* (and also of *Agave asperrima*, Table 5). It has been suggested that certain compounds may be induced by herbivore predators or microbial attackers[33], this could explain the presence of quercetin derivatives in few individuals (three) of *Agave durangensis* from the population Sierra of Registro (Table 3).

The homogeneity in the foliar phenol profiles of individuals of *Agave durangensis* from the population of "Temoaya" represents a strong contrast with that found for the population Sierra of Registro. Assumed as *Agave durangensis*, the individuals from "Temoaya" showed a profile of only three to four kaempferol glycosides (Table 4). The plants growing in "Temoaya" are in average higher (101.3±38.718 cm) than the plants from Sierra of Registro (78.25±23.686 cm) and have longer leaves (73.7±26.765 cm and 54.795±16.130 cm, respectively), with similar middle-leaf wide (19.2±4.134 cm and 18.3±4.401 cm, respectively) and similar spine length (4.5±1.08 and 4.2±1.10 cm, respectively). Both populations (Sierra of Registro and "Temoaya") are separated one from each other by around 50 Km, each with different environmental conditions (Table 1). This could explain the two classes of profiles found in one and another population, since it has been stated that the biosynthesis and accumulation of secondary metabolites depends on highly regulated processes, requiring, among others, environment-specific controls[34]. However, it has been reported that enzymes catalyzing modification reactions of simple flavonoids generally exhibit high substrate specificity, implying that many reactions proceed in a defined sequential order, which seems to be specific for each plant species[35]; then, according to this and to the PCO analysis, the foliar phenol compositions of both populations are different in such a way that each could represent an independent taxon (Fig. 1).

González-Elizondo *et al*.[10] report *Agave angustifolia*, *A. wocomahi*, *A. shrevei* ssp. *magna* and *A. maximiliana* occurring also in Southern Durango. The samples of "Temoaya" could belong to some of those species; however their morphological attributes do not correspond to those given by[10] for each of mentioned species.

Table 4: Individual distribution of foliar flavonoid compounds of *Agave durangensis* from "Temoaya"

Comp.	Retention time (min)	Chem. Ident.	500	501	502	503	504	505	506	507	508	509	510	511	512	513	514	515	516	517	518	519	520	521	522
F1	18.227±0.093	KG	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
F2	18.865±0.00	KG	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
F3	19.598±0.035	KG	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
F4	22.489±0.042	KG	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
F5	23.326±0.089	KG	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	1	0	0	0
F6	24.438± 0.00	KG	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
F7	25.142±0.00	QG	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
F8	27.067±0.00	QG	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
F9	27.710±0.060	KG	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
F10	28.313±0.014	KG	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
F11	27.623±0.051	K3,7OG	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
F12	29.955±0.104	KG	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
F13	29.969±0.090	KAcG	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
F14	31.610±0.056	KG	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
F15	31.434±0.131	KG	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
F16	32.506±0.126	KG	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	0	0	0	0	0	0	0	0
F17	33.238±0.095	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
F18	33.609±0.051	KG	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
F19	34.937±0.086	KRhaG	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
F20	34.720±0.00	QA	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
F21	35.603±0.154	KG	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
F22	35.445±0.046	KG	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
F23	37.148±0.169	KG	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
F24	38.191±0.030	KRh	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
F25	37.198±0.096	KG	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
F26	38.577±0.236	KG	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
F27	38.874±0.00	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
F28	40.004±0.120	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
F29	40.074±0.00	KG	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
F30	40.924±0.00	KG	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
F31	48.528±0.057	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
F32	50.385±0.161	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

*: Mean and standard deviation; KG: Kaempferol Glycoside; QG: Quercetin Glycoside; K3,7OG: Kaempferol-3,7-O-diglucoside; KAcG: Kaempferol-3-O-[6-acetylglucoside]-7-O-glucoside; KRhG: Kaempferol-3-O-[rhamnosyl(1-6) glucoside]; QA: Quercetin-3-O-arabinoside; KRh: Kaempferol-3-O-rhamnoside. 1: Present; 0: Absent

Table 5: Individual distribution of foliar flavonoid compounds of *Agave asperrima*

Comp.	Retention time (min)	Chem. Ident.	301	302	303	304	305	306	307	308	309	310	311	313	314	315	317	319	320	321	322	323	324	325	326
F1	18.227±0.093	KG	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
F2	18.865±0.00	KG	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
F3	19.598±0.035	KG	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
F4	22.489±0.042	KG	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
F5	23.326±0.089	KG	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
F6	24.438±0.00	KG	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
F7	25.142±0.00	QG	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
F8	27.067±0.00	QG	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
F9	27.710±0.060	KG	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
F10	28.313±0.014	KG	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
F11	27.623±0.051	K3,7OG	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
F12	29.955±0.104	KG	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
F13	29.969±0.090	KAcG	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
F14	31.610±0.056	KG	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
F15	31.434±0.131	KG	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
F16	32.506±0.126	KG	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
F17	33.238±0.095	KG	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
F18	33.609±0.051	KG	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
F19	34.937±0.086	KRhG	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
F20	34.720±0.00	QA	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
F21	35.603±0.154	KG	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
F22	35.445±0.046	KG	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
F23	37.148±0.169	KG	0	0	0	1	0	0	0	0	1	0	0	0	1	1	0	0	1	1	1	0	1	1	1
F24	38.191±0.030	KRh	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
F25	37.198±0.096	KG	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
F26	38.577±0.236	KG	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
F27	38.874±0.00	KG	0	0	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Table 5: Continued

F28	40.004±0.120	KG	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
F29	40.074±0.00	KG	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
F30	40.924±0.00	KG	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
F31	48.528±0.057	KG	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
F32	50.385±0.161	KG	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

*: Mean and standard deviation; KG: Kaempferol Glycoside; QG: Quercetin Glycoside; K3,7OG: Kaempferol-3,7-O-diglucoside; KAcG: Kaempferol-3-O-[6-acetylglucoside]-7-O-glucoside; KRhG: Kaempferol-3-O-[rhamnosyl(1-6) glucoside]; QA: Quercetin-3-O-arabinoside; KRh: Kaempferol-3-O-rhamnoside

Table 6: Individual distribution of flavonoid compounds of *Dasylirion* sp.

Comp.	Retention time (min)	Chem. ident.	Dsy1	Dsy2	Dsy3	Dsy4
F1	18.227±0.093	KG	0	0	0	0
F2	18.865±0.00	KG	0	0	0	0
F3	19.598±0.035	KG	0	0	0	0
F4	22.489±0.042	KG	0	0	0	0
F5	23.326±0.089	KG	0	0	0	0
F6	24.438±0.00	KG	0	0	0	0
F7	25.142±0.00	QG	0	0	0	0
F8	27.067±0.00	QG	0	0	0	0
F9	27.710±0.060	KG	0	0	0	0
F10	28.313±0.014	KG	0	0	0	0
F11	27.623±0.051	K3, 7OG	0	0	0	0
F12	29.955±0.104	KG	1	1	1	1
F13	29.969±0.090	KAcG	0	0	0	0
F14	31.610±0.056	KG	1	1	1	1
F15	31.434±0.131	KG	0	0	0	0
F16	32.506±0.126	KG	0	0	0	0
F17	33.238±0.095	0	1	1	1	1
F18	33.609±0.051	KG	0	0	0	0
F19	34.937±0.086	KRhaG	0	0	0	0
F20	34.720±0.00	QA	0	0	0	0
F21	35.603±0.154	KG	1	1	1	1
F22	35.445±0.046	KG	0	0	0	0
F23	37.148±0.169	KG	0	0	0	0
F24	38.191±0.030	KRh	0	0	0	0
F25	37.198±0.096	KG	0	0	0	0
F26	38.577±0.236	KG	0	0	0	0
F27	38.874±0.00	0	0	0	0	0
F28	40.004±0.120	0	1	1	1	1
F29	40.074±0.00	KG	0	0	0	0
F30	40.924±0.00	KG	0	0	0	0
F31	48.528±0.057	0	1	1	1	1
F32	50.385±0.161	0	1	1	1	1

*: Mean and standard deviation; KG: Kaempferol Glycoside; QG: Quercetin Glycoside; K3,7OG: Kaempferol-3,7-O-diglucoside; KAcG: Kaempferol-3-O-[6-acetylglucoside]-7-O-glucoside; KRhG: Kaempferol-3-O-[rhamnosyl(1-6) glucoside]; QA: Quercetin-3-O-arabinoside; KRh: Kaempferol-3-O-rhamnoside. 1: Present; 0: Absent

The foliar phenol profiles of all individuals identified, on a morphological basis, as *Agave asperrima* (samples 300-333) showed a very simple foliar phenol profile. The individual profiles were very homogeneous. The patterns were formed for only one or two among three different kaempferol glycosides (Table 5). The PCO analysis clearly separates the samples of *A. asperrima* from all the other individuals (Fig. 1).

None kaempferol glycoside derivative was found as part of foliar phenol profile of *Dasylirion* sp., but one quercetin glycoside, one luteolin glycoside, probably one gossypetin glycoside and four unidentified flavones glycosides were detected (Table 6).

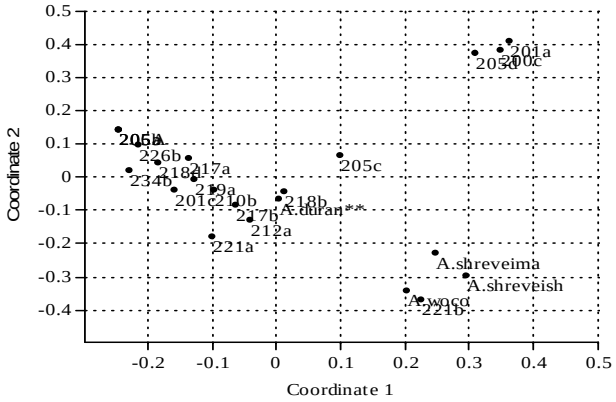

Fig. 2: Results of Principal Coordinates Analysis comparing foliar flavonoid profiles for juvenile samples of *Agave durangensis* from Sierra de Registro (200c-234b), *Agave durangensis* from Botanico Garden, UNAM (*A. duran***), *A. shrevei* subsp. *shrevei* (*A. shrevei* sh), *A. shrevei* subsp. matapensis (*A. shrevei* ma) and *A. wocomahi* (*A. woco*)

The PCO analysis place *Dasylirion* sp., which belongs to family Nolinaceae[9], clearly separated from *Agave* (Fig. 1).

The foliar phenol profiles of juvenile samples of *Agave durangensis* were more complex and variable than those belonging to adults (Table 7). A total of 28 different kaempferol glycosides were found and from three to eleven could be present in any sample. Compounds J10, J14 and J22 (Table 7) are shared with adults. However, a considerable difference exists between juvenile and adult profiles. This difference suggests that, contrary to reported for others species of plants by Abou-Zaid and Nozzolillo[36] and Almaraz-Abarca *et al.*[15], who did not find any age-related variability in foliar flavonoid composition, the qualitative expression of *A. durangensis* foliar flavonoids is not defined from the juvenile stage. The role of all these compounds in the growth and development processes of this species is left for determining.

The phenol foliar profile of juvenile sample of *A. durangensis* donated by the Botanic Garden, UNAM, was formed by four kaempferol glycoside derivatives,

Table 7: Individual distribution of flavonoid compounds of juvenile plants of *Agave durangensis*, *A. shrevei* subsp. *shrevei*, *A. shrevei* subsp. *matapensis*, and *A. wocomah*

Comp.	Retention time (min)*	Chem. Ident.	200c	201a	201c	205a	205b	205c	205d	206a	210b	212a	217a	217b	218b	218d	219a	221a	221b	226b	234b	*A. duran***	*A. shrevei* sh	*A. shrevei* ma	*A. woco*
J1	13.216±0.00	KG	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
J2	15.857±0.098	KG	1	1	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
J3	17.126±0.069	KG	1	1	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
J4	20.214±0.00	KG	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
J5	21.353±0.132	KG	1	1	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0
J6	21.838±0.084	KG	0	1	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
J7	24.585±0.00	KG	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0
J8	27.108±0.79	KG	0	0	0	0	0	0	0	0	0	1	0	1	1	0	0	0	1	0	0	0	0	0	1
J9	28.149±0.110	KG	0	0	0	0	0	0	0	0	0	1	0	1	0	0	0	1	1	0	0	0	1	1	1
J10	27.704±0.040	K3, 7OG	0	0	1	1	1	0	0	1	0	1	1	1	1	1	1	1	0	1	1	0	0	0	0
J11	29.352±0.00	KG	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
J12	30.892±0.088	KG	0	0	1	0	0	1	0	0	0	1	0	0	1	0	0	0	1	0	0	1	1	0	0
J13	30.965±0.00	KG	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
J14	31.624±0.027	KG	0	0	1	1	1	0	0	1	1	1	1	1	1	1	1	0	1	1	1	0	0	0	0
J15	32.983±0.31	KG	0	0	1	0	0	0	0	0	1	1	1	1	0	1	1	1	1	0	1	0	0	0	1
J16	34.060±0.044	KG	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	0	0	1	0	1	0	1	0
J17	35.064±0.032	KG	0	0	1	0	0	0	0	0	0	0	0	1	0	1	0	0	0	0	0	1	0	0	0
J18	35.911±0.054	KG	0	0	0	0	0	1	0	0	0	0	1	0	1	0	0	0	1	0	0	0	0	0	0
J19	36.685±0.00	FG	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0
J20	36.806±0.138	KG	0	0	0	0	0	0	0	0	0	1	1	1	1	1	1	1	0	0	0	0	0	0	0
J21	36.011±0.00	K3-OG	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
J22	38.269±0.080	KRh	0	0	1	1	1	1	0	1	0	1	1	1	0	1	0	0	0	1	1	0	0	0	0
J23	37.466±0.00	FG	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
J24	37.838±0.047	KG	0	0	0	0	0	0	1	0	0	1	1	1	0	1	0	0	0	0	0	0	0	0	0
J25	38.461±0.133	KG	0	0	0	0	0	1	1	0	0	1	1	0	0	0	1	0	0	0	0	0	0	0	0
J26	40.620±0.054	KG	0	0	1	0	0	0	0	0	1	0	0	0	0	0	1	0	0	1	0	0	0	0	0
J27	42.191±0.094	KG	0	0	0	0	0	1	1	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0
J28	43.622±0.132	KG	0	0	0	0	0	0	0	0	0	0	1	1	0	1	0	0	0	0	0	0	0	0	0
		Total	5	5	8	3	3	5	6	3	4	10	9	11	8	8	7	5	5	5	4	4	3	3	4

*: Mean and standard deviation; **: Sample donated by Jordan Botánico UNAM; KG: Kaempferol Glycoside; K3, 7OG: Kaempferol-3,7-O-diglucoside; K3-OG: KRh: Kaempferol-3-O-rhamnoside. 1: Present; 0: Absent

all also present in the juvenile samples of *A. durangensis* collected and analyzed (Table 7). That donated sample is situated among most of the *A. durangensis* samples collected from Sierra of Registro by the PCO analysis. Other three groups are distinguished, each formed by one to three individuals; these groups could represent different chemotypes (Fig. 2).

Agave wocomahi, which belongs to the same section *Ditepalae* than *Agave durangensis*[3], showed profiles formed by four kaempferol derivatives (J8, J9, J13 and J15), all, except J13, are also present in juvenile *A. durangensis* samples (Table 7). The PCO analysis places this species close to the juvenile sample 221b of *A. durangensis*.

Concerning to the foliar phenol profile of *A. shrevei* subsp. *shrevei* and *A. shrevei* subsp. *matapensis* (belonging also to the same section *Ditepalae*), their profiles were different to that of *A. wocomahi* and that of *A. durangensis*. They were formed by three kaempferol glycoside derivative, from which one (J9) was common to both (Table 7). The PCO analysis of foliar profiles of juvenile samples groups together both subspecies of *A. shrevei* (Fig. 2).

CONCLUSION

The foliar and pollen phenol compositions of *Agave durangensis* are rich in glycosides derivatives of the flavonol kaempferol. Actually, this kind of compounds domain in all species of *Agave* studied. These taxa could be distinguished by the foliar phenol components and some species-specific patterns could be discerned. This reveals the flavonoid foliar profiles as valuable specific chemical markers in *Agave*.

In spite of the intrapopulation variability detected within the population of *Agave durangensis* from Sierra of Registro, the presence of specific flavonoid components in the leaves of each analyzed population (Sierra of Registro and "Temoaya") suggest the recognition of two independent taxonomic entities within this morphologically variable group.

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