

## Collecting Venezuelan *Stylosanthes* species

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### Abstract

A report on recent activities to collect representative germplasm samples of *Stylosanthes* species native to Venezuela is presented. The main objective was to obtain original seeds for phylogenetic studies and, at the same time, to contribute to safeguarding *Stylosanthes* diversity, in view of the increasing danger of genetic erosion. Seeds of 33 samples, comprising all 11 Venezuelan *Stylosanthes* species, were collected: *S. angustifolia*, *S. capitata*, *S. gracilis*, *S. guianensis*, *S. hamata*, *S. humilis*, *S. scabra* and *S. viscosa* plus the endemic and little-known *S. falconensis*, *S. sericeiceps* and *S. venezuelensis*. Populations of the latter 3 species were small and difficult to locate; these species must be considered endangered and require further attention by genetic resources specialists. Furthermore, assessment of their agronomic and forage potential, in particular, *S. falconensis* and *S. sericeiceps*, is warranted.

### Resumen

Se presenta un informe sobre unas recientes actividades para recolectar muestras representativas de germoplasma de las especies venezolanas de *Stylosanthes*. El objetivo principal fue obtener semillas originales para ser usadas en estudios filogenéticos y, al mismo tiempo, contribuir a salvaguardar la diversidad de *Stylosanthes*, en vista del creciente peligro de erosión genética que afecta también a este género. Como resultado, se recolectaron un total de 33 muestras de semillas que comprenden las 11 especies venezolanas de *Stylosanthes*: *S. angustifolia*, *S. capitata*, *S. gracilis*, *S. guianensis*, *S. hamata*, *S. humilis*, *S. scabra*, *S. viscosa* y las endémicas y poco conocidas *S. falconensis*, *S. sericeiceps* y *S. venezuelensis*. Las poblaciones de las tres últimas especies fueron difíciles de localizar y pequeñas; por lo tanto, estas especies deben ser consideradas como en peligro y requieren una mayor atención por parte de los especialistas en recursos genéticos. Además ameritan ser evaluadas respecto a su potencial agronómico y forrajero, en particular *S. falconensis* y *S. sericeiceps*.

### Introduction

*Stylosanthes* is considered one of the most important tropical forage legume genera (Burt et al. 1983; Chakraborty 2004). Primarily because most of its species are adapted to infertile soils and drought, it has received considerable attention by tropical pasture scientists and is well represented in the major forage genetic resources collections (Stace and Edye 1984; Cook et al. 2005).

Venezuela is an important center of diversification of *Stylosanthes*; 11 species have been reported, 3 of them being endemic to the country (Calles and Schultze-Kraft 2010a). During 1973–1986, a significant collection of more than 600 accessions of Venezuelan *Stylosanthes* germplasm was assembled (Schultze-Kraft 1991).

A recent study of diversity of Venezuelan *Stylosanthes* (Calles 2012) has shown the convenience of molecular marker analyses to further describe genetic variation and species boundaries and relationships. For this, original seed samples (those collected at the original sites) should be used, rather than material that has undergone various cycles of seed multiplication in gene bank plant nurseries, with the concomitant risk that genetic integrity of the accession may be distorted (Chebotar et al. 2003).

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This risk was recently pointed out by Garcia et al. (2013) for several *Stylosanthes* species, which usually are considered to be predominantly self-pollinating. They reported outcrossing rates of up to 31%. The Venezuelan diversity study also highlighted the increasing destruction of native habitats due to land use changes in the country, and subsequent endangerment of *Stylosanthes* populations.

This paper reports on recent (2006–2008) collecting activities in Venezuela to obtain representative germplasm samples of all 11 Venezuelan *Stylosanthes* species, with the objective of: (1) making original seeds available for phylogenetic analyses; and, at the same time, (2) safeguarding *Stylosanthes* diversity in view of the danger of genetic erosion.

## Methodology

Three collection sites for each Venezuelan *Stylosanthes* species were predefined, based on exhaustive herbarium studies using specimens from 13 international and 22 Venezuelan herbaria (Calles and Schultze-Kraft 2010a). In order to cover as much intraspecific diversity as possible, the sites identified were in environments as contrasting as possible and/or with the largest possible geographic distance between them.

At each site, we collected as many mature seeds as possible from the target populations; if possible, 10 or more plants per population were sampled and the seeds bulked. Soil samples from the 0–10 cm horizon were taken and analyzed to determine texture, pH and phosphorus (P) content.

Geographical location and elevation were recorded with a GPS device and later refined using the geographical information program Google Earth (<http://earth.google.com>). Climatic information (i.e. mean annual rainfall, number of dry months and annual mean temperature) at collection sites was obtained from the software DIVA-GIS (Hijmans et al. 2005); a month with less than 60 mm of rainfall was considered dry. Vegetation/ecozone classification at collection sites was performed by locating the collection sites manually on the vegetation map of Venezuela (Huber and Alarcón 1988).

In compliance with national regulations governing access to Venezuelan plant genetic resources, a collection permit was obtained from the Ministry of Environmental Affairs (MINAMB, its Spanish acronym).

## Results and Discussion

### General

A total of 33 seed samples were collected. Except for *S. capitata*, *S. scabra* and *S. sericeiceps* (4 samples each), *S. viscosa* (2 samples) and *S. venezuelensis* (1 sample), 3 samples of each Venezuelan species were obtained as intended. The samples were incorporated in the collection of the Venezuelan Genebank for Forage Legumes (BGLFV, its Spanish acronym) at INIA-Anzoátegui, El Tigre; it is intended to deposit a duplicate at the Genetic Resources Program of CIAT, Cali, Colombia. Furthermore, at least 2 voucher herbarium specimens from plants with flowers and fruits were taken from each population and deposited at the Herbario Nacional de Venezuela (VEN). Passport data of the collected material are provided in Table 1.

### Climate, soil and vegetation

Collections were made from an elevation range of 10–1,871 masl, with the only exception being a site at 2,481 masl, where a population of *S. sericeiceps* (CAL014) was collected in the Andes region, municipality of Mérida (Table 1). Most sampled populations were in areas with mean annual rainfall (MAR)  $\geq 1,000$  mm (Table 2) and with a dry season of up to 6 months. Interestingly, all populations of *S. falconensis* and one *S. viscosa* population (CAL005) were collected in areas with MAR as low as 781 and 312 mm, respectively. Moreover, at the collection site of sample CAL005, no month registered more than 60 mm rainfall. Most species were collected in areas with annual mean temperature (AMT) between 20 and 28 °C (Table 2), but it is noteworthy that some samples of *S. guianensis* (CAL030, CAL032), *S. hamata* (CAL012), *S. scabra* (CAL028, CAL031) and all samples of *S. sericeiceps* were collected in areas with AMT  $< 20$  °C.

Savanna was the original vegetation type, where the largest number of samples originated (12 samples), followed by: thorn forest and semi-deciduous forest (5 samples each); deciduous forest (4 samples); littoral vegetation and evergreen forest (3 and 2 samples, respectively); and gallery and cloud forests (1 sample each). In many cases, habitat destruction, as a consequence of land use changes, was observed.

**Table 1.** Passport data of *Stylosanthes* germplasm samples collected in Venezuela.

Collector number	Species	Collectors	State	km loc.	Locality	Lat. (°)	Long. (°)	Elev. (m)	Date	Herbarium specimen
CAL001	<i>S. venezuelensis</i>	Calles and Schultze-Kr.	Distrito		Caracas	10.495	-66.891	895	10.02.06	<i>Calles et al. 1001</i>
CAL002	<i>S. hamata</i> (L.) Taub.	T. Calles, R. Schultze-Kraft, O. Guenni, Y. Espinoza	Capital							
CAL003	<i>S. angustifolia</i> Vogel	T. Calles, E.M. Walle	Falcón	1 NW	La Luisa	10.868	-68.314	202	08.04.07	<i>Calles &amp; Walle 1014</i>
CAL004	<i>S. angustifolia</i> Vogel	T. Calles, E. Colmenares	Guárico	31 SE	Calabozo	8.703	-67.297	78	25.04.07	<i>Calles &amp; Colmenares 1015</i>
CAL005	<i>S. viscosa</i> (L.) Sw.	T. Calles, E. Colmenares	Apure		Fundo Picachón	7.022	-67.668	49	26.04.07	<i>Calles &amp; Colmenares 1016</i>
CAL006	<i>S. hamata</i> (L.) Taub.	T. Calles, J. García	Sucre	0.5 SW	Tacarigua	10.569	-64.171	10	06.05.07	<i>Calles &amp; García 1017</i>
CAL007	<i>S. falconensis</i> Calles and Schultze-Kr.	T. Calles	Sucre		Cumaná	10.430	-64.194	18	12.05.07	<i>Calles 1018</i>
CAL008	<i>S. falconensis</i> Calles and Schultze-Kr.	T. Calles	Falcón	0.6 SW	Carrizalito	11.130	-69.757	1,169	23.07.07	<i>Calles 1019</i>
CAL009	<i>S. falconensis</i> Calles and Schultze-Kr.	T. Calles	Falcón	0.4 NW	La Peña	11.108	-69.750	910	24.07.07	<i>Calles 1020</i>
CAL010	<i>S. humilis</i> Kunth	T. Calles	Falcón		Cucaire	11.124	-69.760	1,165	25.07.07	<i>Calles 1021</i>
CAL011	<i>S. sericeiceps</i> S.F. Blake	T. Calles	Portuguesa	7 NW	Guanare	9.066	-69.810	259	14.12.07	<i>Calles 1022</i>
CAL012	<i>S. hamata</i> (L.) Taub.	T. Calles	Mérida	3 NW	Lagunillas	8.515	-71.412	1,363	17.12.07	<i>Calles 1023</i>
CAL013	<i>S. sericeiceps</i> S.F. Blake	T. Calles	Mérida		La González	8.504	-71.326	819	17.12.07	<i>Calles 1024</i>
CAL014	<i>S. sericeiceps</i> S.F. Blake	T. Calles	Mérida		La González	8.502	-71.320	762	17.12.07	<i>Calles 1025</i>
CAL015	<i>S. sericeiceps</i> S.F. Blake	T. Calles	Mérida	4 NW	El Morro	8.471	-71.209	2,481	18.12.07	<i>Calles 1026</i>
CAL016	<i>S. humilis</i> Kunth	T. Calles, R. Schultze-Kraft	Guárico		Palo Seco	9.057	-67.238	141	06.02.08	<i>Calles &amp; Schultze-Kraft 1027</i>
CAL017	<i>S. gracilis</i> Kunth	T. Calles, R. Schultze-Kraft	Guárico	6 NW	El Mejo	8.620	-66.434	139	06.02.08	<i>Calles &amp; Schultze-Kraft 1028</i>
CAL018	<i>S. angustifolia</i> Vogel	T. Calles, R. Schultze-Kraft	Guárico	6 NW	El Mejo	8.620	-66.434	139	06.02.08	<i>Calles &amp; Schultze-Kraft 1029</i>
CAL019	<i>S. capitata</i> Vogel	T. Calles, R. Schultze-Kraft	Guárico	17 S	Santa Rita	7.974	-66.238	53	06.02.08	<i>Calles &amp; Schultze-Kraft 1030</i>
CAL020	<i>S. guianensis</i> (Aubl.) Sw.	T. Calles, R. Schultze-Kraft	Anzoátegui	6 NW	Santa Juana	8.946	-64.758	214	07.02.08	<i>Calles &amp; Schultze-Kraft 1031</i>
CAL021	<i>S. scabra</i> Vogel	T. Calles, R. Schultze-Kraft	Anzoátegui	6 NW	Santa Juana	8.946	-64.758	214	07.02.08	<i>Calles &amp; Schultze-Kraft 1032</i>
CAL022	<i>S. gracilis</i> Kunth	T. Calles, R. Schultze-Kraft	Anzoátegui	6 NE	Caracol	8.919	-64.608	343	07.02.08	<i>Calles &amp; Schultze-Kraft 1033</i>
CAL023	<i>S. capitata</i> Vogel	T. Calles, R. Schultze-Kraft	Anzoátegui	14 NE	Santa Clara	8.610	-64.557	191	08.02.08	<i>Calles &amp; Schultze-Kraft 1034</i>
CAL024	<i>S. scabra</i> Vogel	T. Calles, R. Schultze-Kraft	Sucre	5 SW	Santa Fe	10.232	-64.424	248	09.02.08	<i>Calles &amp; Schultze-Kraft 1035</i>
CAL025	<i>S. capitata</i> Vogel	T. Calles, R. Schultze-Kraft	Monagas	4 S	Chaguaramas	8.690	-62.774	71	11.02.08	<i>Calles &amp; Schultze-Kraft 1038</i>
CAL026	<i>S. viscosa</i> (L.) Sw.	T. Calles, R. Schultze-Kraft	Bolívar		La Ceiba	7.678	-63.508	143	11.02.08	<i>Calles &amp; Schultze-Kraft 1040</i>
CAL027	<i>S. capitata</i> Vogel	T. Calles, R. Schultze-Kraft	Bolívar		Cardozo	8.000	-63.546	106	12.02.08	<i>Calles &amp; Schultze-Kraft 1042</i>
CAL028	<i>S. humilis</i> Kunth	T. Calles, R. Schultze-Kraft	Bolívar		Cardozo	8.000	-63.546	106	12.02.08	<i>Calles &amp; Schultze-Kraft 1043</i>
CAL029	<i>S. scabra</i> Vogel	T. Calles, T. Beuchelt	Táchira		Lobatera	7.933	-72.241	1,010	19.02.08	<i>Calles &amp; Beuchelt 1044</i>
CAL030	<i>S. sericeiceps</i> S.F. Blake	T. Calles, T. Beuchelt	Mérida	1 SW	Higuerones	8.522	-71.282	868	20.02.08	<i>Calles &amp; Beuchelt 1045</i>
CAL031	<i>S. guianensis</i> (Aubl.) Sw.	T. Calles, T. Beuchelt	Mérida	5 S	Mérida	8.535	-71.180	1,871	20.02.08	<i>Calles &amp; Beuchelt 1046</i>
CAL032	<i>S. scabra</i> Vogel	T. Calles, T. Beuchelt	Lara	1 SE	Humocaro Alto	9.599	-69.982	1,024	23.02.08	<i>Calles &amp; Beuchelt 1047</i>
CAL033	<i>S. guianensis</i> (Aubl.) Sw.	T. Calles, T. Beuchelt	Lara	1 SE	Humocaro Alto	9.599	-69.982	1,024	23.02.08	<i>Calles &amp; Beuchelt 1048</i>
CAL033	<i>S. gracilis</i> Kunth	T. Calles, T. Beuchelt	Zulia	3 S	La Raya	9.848	-70.878	48	27.02.08	<i>Calles &amp; Beuchelt 1 050</i>

All species were growing on extremely acid to slightly acid soils (Table 3), except for *S. falconensis* and *S. venezuelensis* (slightly alkaline to neutral). It is noteworthy that *S. angustifolia*, *S. capitata*, *S. gracilis* [formerly known as *S. guianensis* var. *gracilis* (Kunth) Vogel; Calles and Schultze-Kraft 2010b] and *S. humilis* consistently came from very strongly acid soils (classification according to SSDS 1993). Soil P content was low in most cases, except for *S. guianensis*, *S. hamata* and *S. sericeiceps* (medium to high P content). It was significant that only *S. scabra* was growing on a heavier textured soil (clay loam).

In general, climatic and soil conditions at collection sites are in agreement with what is known about ecological conditions where *Stylosanthes* species occur (Stace and Edye 1984). We draw attention to the very high site elevation of sample CAL014 (the endemic *S. sericeiceps*; above 2,400 masl; Figures 1A, B & C) and the very low rainfall at the site of CAL005 (*S. viscosa*; GIS-DIVA calculated MAR of 312 mm). Both are quite unusual for *Stylosanthes* species and germplasm from those sites might be a particularly interesting resource for selection and/or breeding projects concerned with cold- and drought-tolerance, respectively.

**Table 2.** Climatic characteristics (ranges) and vegetation types at collection sites of Venezuelan *Stylosanthes* species.

Species	MAR <sup>1</sup> (mm)	Dry months <sup>2</sup> (No.)	AMT <sup>3</sup> (°C)	Vegetation type <sup>4</sup> (Collector number)
<i>S. angustifolia</i>	1,297–1,868	4–5	26.7–27.9	SA (003, 017), GF (004)
<i>S. capitata</i>	943–1,512	4–6	26.2–27.8	SA (018, 022, 024), SF (026)
<i>S. falconensis</i>	781	5	23.5	DF (007, 008, 009)
<i>S. gracilis</i>	1,035–1,378	3–5	26.5–26.8	SA (016, 021, 033)
<i>S. guianensis</i>	1,052–1,214	3–5	15.8–26.9	SA (019), CF (030), EF (032)
<i>S. hamata</i>	1,089–1,141	2–5	15.8–26.6	LV (002, 006), TF (012)
<i>S. humilis</i>	943–1,597	4–6	27.3–27.8	SF (010, 027), SA (015)
<i>S. scabra</i>	925–1,223	3–5	18.8–26.9	SA (020), SF (023), TF (028), EF (031)
<i>S. sericeiceps</i>	1,088–1,127	3–4	14.5–16.4	TF (011, 013, 029), DF (014)
<i>S. venezuelensis</i>	1,003	5	20.0	SF (001)
<i>S. viscosa</i>	312–1,164	4–12	27.1–27.4	LV (005), SA (025)

<sup>1</sup>Mean annual rainfall; <sup>2</sup>months with <60 mm rainfall; <sup>3</sup>annual mean temperature; <sup>4</sup>according to Huber and Alarcón (1988): SA, savanna; GF, gallery forest; SF, semi-deciduous forest; DF, deciduous forest; CF, cloud forest; EF, evergreen forest; LV, littoral vegetation; TF, thorn forest.

**Table 3.** Some soil characteristics (mean values) at collection sites of Venezuelan *Stylosanthes* species.

Species	Texture <sup>1</sup> (Collector number)	pH <sup>2</sup> (±SD)	Phosphorus <sup>3</sup> (±SD) (ppm)
<i>S. angustifolia</i>	loamy sand (003), sand (004), loam (017)	4.8 (0.7)	6.3 (5.8)
<i>S. capitata</i>	sand (018, 022), sandy loam (024), loamy sand (026)	4.4 (0.2)	4.0 (0.0)
<i>S. falconensis</i>	sandy loam (007, 008, 009)	7.4 (0.3)	5.7 (4.6)
<i>S. gracilis</i>	loam (016), sand (021)	4.6 (0.6)	3.0 (0.0)
<i>S. guianensis</i>	loamy sand (019, 030), silt loam (032)	5.9 (1.0)	43.3 (68.2)
<i>S. hamata</i>	sandy loam (002, 006), loam (012)	6.3 (2.0)	34.3 (46.5)
<i>S. humilis</i>	sandy loam (010), silt loam (015), loamy sand (027)	4.6 (0.2)	4.7 (2.1)
<i>S. scabra</i>	loamy sand (020, 023), clay loam (028, 031)	5.7 (1.3)	11.0 (13.6)
<i>S. sericeiceps</i>	sandy loam (011, 013), loam (014), loamy sand (029)	6.2 (0.9)	108 (74.2)
<i>S. venezuelensis</i>	sandy loam (001)	7.3	15.0
<i>S. viscosa</i>	sandy loam (005), loamy sand (025)	4.8 (0.4)	3.5 (0.7)

Methodology: <sup>1</sup>Bouyoucos; <sup>2</sup>Water 1:1; <sup>3</sup>Mehlich I.



**Figure 1.** *Stylosanthes sericeiceps* (sample CAL014): **A** collection site; **B** overgrazed old plant; **C** flowering branches. *Stylosanthes falconensis* (sample CAL009): **D** collection site; **E** adult plant; **F** flowering branch. *Stylosanthes venezuelensis* (sample CAL001): **G** plants at collection site; **H** flowering branch. Photos A, B, C, G & H by R. Schultze-Kraft; D–F by T. Calles.

### Endemic species

During our collecting activities we observed that the 3 endemic *Stylosanthes* species (*S. falconensis*, *S. sericeiceps* and *S. venezuelensis*) have very restricted distributions. Populations occurred in areas, where human intervention is negatively affecting their habitats, and were rather small and difficult to find. All 3 species must be considered rare. At collection sites, *S. falconensis* (a new species, closely related to *S. hamata*; Calles and Schultze-Kraft 2010c; Figures 1D, E & F) showed vigorous growth and plants were evidently grazed by livestock. This suggests that the forage potential of this species is worthy of evaluation. We observed apparent fertility problems at all 3 sites for that species; while plants were flowering profusely, there was very little seed set. The same was observed with 1 of the 3 *S. sericeiceps* populations (CAL011).

The third endemic species, *S. venezuelensis* (closely related to *S. scabra*; Calles and Schultze-Kraft 2009; Figures 1G & H), is particularly rare. In spite of our intensive search, we found only 1 small population in strongly intervened vegetation within the Caracas urban area.

Continued collecting of germplasm of these endemic species is required in order to safeguard their genetic diversity. In the case of *S. venezuelensis*, protection measures might be needed to avoid the extinction of the species. An initial step would be to multiply the collected material and disperse the seed into the species' native habitats.

### Conclusion and Outlook

Original seed of all Venezuelan *Stylosanthes* species is now available for the intended phylogenetic studies. With the material collected, we might have made only a minor contribution to increased genetic diversity within the 8 non-endemic species, since, with the exception of *S. angustifolia*, they had been fairly well sampled in the 1970s and 1980s (Schultze-Kraft 1991). Rather, the significance of the collection reported here lies in the fact that germplasm of the 3 endemic species, *S. falconensis*, *S. sericeiceps* and *S. venezuelensis*, is available for the first time. These species are rare and must be considered endangered. Efforts to collect more material to safeguard genetic diversity are indicated, particularly in view of the destruction of the species' habitats.

Assessment of the agronomic and forage potential of the endemic species, particularly of *S. falconensis* and *S. sericeiceps*, is suggested.

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