Morphological variation and classification of field-grown *Stylosanthes seabrana* and *S. scabra*

R.A. DATE, P. I. JANSEN, B. MESSER AND D.A. EAGLES
CSIRO, Division of Tropical Crops and Pastures, St Lucia, Queensland, Australia

Abstract

Seventy-eight *Stylosanthes seabrana* and *S. scabra* lines were grown at CSIRO Lansdown Research Station, north Queensland and 32 morphological and agronomic characteristics were measured and used as attributes to define similarity groups using the numerical classification program PATN.

At the 5-Group level, groups were mostly homogeneous by species. Terminal leaflet shape and terminal leaflet apex shape, the presence or absence of leaflet and stem hairs, the presence or absence of inflorescence bristles, the presence or absence of stipule horn lateral bristles and stipule horn terminal bristles were the most useful attributes defining groups.

Introduction

Stylosanthes seabrana was described by Maass and Mannetje (2002) but was recognised in the 1980s by CSIRO's Plant Introduction group at the Davies Laboratory, Townsville, and by CIAT's Genetic Resources group in Colombia. In both cases, the 'new' species was described as being intermediate in morphology and growth between *Stylosanthes hamata* and *Stylosanthes scabra*. Jansen and Edye (1996) referred to the 'new' species as *S.* sp.aff. *S. scabra* and Maass (1989) as 'cf. scabra-type'. Jansen and Edye (1996) reported that the morphological attributes leaf and stem bristles, stem hairs and seed colour would be useful attributes in separating *S. seabrana* from *S. hamata* and *S. scabra*. Two accessions of *S. seabrana* (commonly called Caatinga Stylo), CPI 92838B and CPI 110361 from their Group 3, were released in 1996 as the commercial cultivars 'Primar' and 'Unica', respectively (Anon 1996).

Early field observations (Date *et al*. 1996; L.A. Edye and R.A. Date, unpublished data) suggested that neither cultivar nodulated effectively with native strains of root nodule bacteria (RNB). Selection of effective and persistent strains of RNB (*Bradyrhizobium*), for the 2 cultivars is reported separately (Date 2010). In a mixed cropping-pasture system, inclusion of Caatinga stylo cv. Unica in the pasture phase improved soil nitrogen in the top 75 cm of soil (Pengelly *et al*. 2004).

Since the release of the new cultivars, an additional 50 accessions of similar legume germplasm have been identified: 20 collected by L.A. Edye and R.A. Date (1994, unpublished data) and recorded as *S. seabrana* (initially, and in retrospect incorrectly, as '*S.* sp.aff. *S. scabra*') in the CSIRO database and 30 from the CIAT germplasm collection recorded as *S. scabra*. Maass (1989) refers to these 30 accessions as 'aff.-scabra-type'. All except 9 of these 50 accessions originated from the state of Bahia, Brazil.

This paper reports early morphological characterisation of these additional accessions using a similar methodology to that of Jansen and Edye (1996).

Materials and methods

Experimental layout

Fifteen of the 16 accessions of *S. seabrana* used by Jansen and Edye (1996), 18 additional *S. seabrana* and 11 *S. scabra* accessions collected by Edye and Date (unpublished data) in 1994, 1 recently identified *S. seabrana* from CSIRO's collection, 30 new accessions of *S. scabra* from

Correspondence: R.A. Date, 55 Molonga Tce, Graceville, Queensland 4075, Australia. E-mail: jmdrad@powerup.com.au

CIAT (Maass aff.-scabra-type), 2 *S. scabra* cultivars, Seca and Siran, and 1 *S. hamata* cv. Amiga were grown at the CSIRO Lansdown Research Station 40 km west of Townsville, northern Queensland. CPI accession numbers and species names as per the CSIRO database in 1994 are listed in Table 1.

Seed was germinated on 1% water agar and the seedlings grown in fine sand in a glasshouse from mid-August 1996 and finally transplanted to the field in October 1996. Plants were grown on an area covered with weed-matting, 10 replications of 10 plants at 50 cm spacing in rows, with 2 m between rows. The plot area was irri-

Table 1. Member number, origin collection numbers and species of *Stylosanthes* accessions grown in rows at Lansdown, north Queensland (see text for explanations).

Member[1]	ATF/CPI[2]	Species[3]	CIAT[4]	State[5]	Type[6]	Region[6]
79	CPI38842	*hamata*	cv. Amiga[7]	ZUL		
59	ATF2328	*scabra*	2076	BA	aff	6
48	ATF2329	*scabra*	2084	BA	aff	6
12	ATF2330	*scabra*	2287	MG	aff	6
50	ATF2331	*scabra*	2364	BA	aff	6
13	ATF2332	*scabra*	2366	BA	aff	5
10	ATF2333	*scabra*	2375	BA	aff	5
15	ATF2334	*scabra*	2376	BA	aff	5
31	ATF2335	*scabra*	2383	BA	aff	5
52	ATF2336	*scabra*	2533	BA	aff	
47	ATF2337	*scabra*	2751	BA	aff	5
65	ATF2338	*scabra*	10031	BA	aff	6
64	ATF2339	*scabra*	10043	BA	aff	4
73	ATF2340	*scabra*	10045	BA	aff	6
39	ATF2341	*scabra*	10046	BA	aff	6
3	ATF2342	*scabra*	10047	BA	aff	6
66	ATF2343	*scabra*	10050	MG	aff	6
45	ATF2344	*scabra*	10052	MG	aff	6
67	ATF2345	*scabra*	10054	MG	aff	6
30	ATF2346	*scabra*	10056	MG	aff	6
23	ATF2347	*scabra*	1057	MG	aff	4
29	ATF2348	*scabra*	10062	MG	aff	4
38	ATF2349	*scabra*	10093	BA	po	6
35	ATF2351	*scabra*	10537	BA	cf	6
19	ATF2352	*scabra*	10726	MG	aff	4
17	ATF2353	*scabra*	10732	MG	aff	4
36	ATF2354	*scabra*	11331	BA	bra	6
41	ATF2355	*scabra*	11340	BA		
11	ATF2356	*scabra*	11341	BA		
42	ATF2357	*scabra*	11343	BA	bra	6
24	ATF2358	*scabra*	11554	BA		
2	ATF2499	*scabra*		BA		
63	ATF2501B	*scabra*		BA		
70	ATF2524	*scabra*		MG		
61	ATF2525	*scabra*		MG		
5	ATF2526	*scabra*		MG		
53	ATF2527	*scabra*		MG		
76	ATF2528	*scabra*		MG		
28	ATF2529	*scabra*		MG		
7	CPI40292	*scabra*	cv. Seca[7]	ZUL		
57	CPI bred line	*scabra*	cv. Siran[7]			
72	ATF2350	*seabrana*	10471	BA	cf	6
4	ATF2507B	*seabrana*		BA		
34	ATF2516	*seabrana*		BA		
27	ATF2517	*seabrana*		BA		
56	ATF2518	*seabrana*		BA		
43	ATF2519	*seabrana*		BA		
62	ATF2520	*seabrana*		BA		
55	ATF2521	*seabrana*		BA		
54	ATF2522	*seabrana*		BA		
21	ATF2523	*seabrana*		BA		
26	ATF2530	*seabrana*		BA		
46	ATF2531	*seabrana*		BA		
60	ATF2532	*seabrana*		BA		
58	ATF2533	*seabrana*		BA		
75	ATF2534	*seabrana*		BA		
1	ATF2535	*seabrana*		BA		
20	ATF2536	*seabrana*		BA		

Member[1]	ATF/CPI[2]	Species[3]	CIAT[4]	State[5]	Type[6]	Region[6]
51	ATF2537	*seabrana*		BA		
25	ATF2539	*seabrana*		BA		
71	ATF2539B	*seabrana*		BA		
18	ATF2540	*seabrana*		BA		
37	ATF2540B	*seabrana*		BA		
49	CPI104710	*seabrana*	10026	BA	cf	6
68	CPI105546B	*seabrana*		BA		
9	CPI110340	*seabrana*	2043	BA	cf	6
16	CPI110341	*seabrana*	2050	BA	cf	6
6	CPI110342	*seabrana*	2070	BA	cf	6
33	CPI110343	*seabrana*	2085	BA	cf	6
22	CPI110361	*seabrana*	cv. Unica[7] 10033	BA	cf	6
69	CPI110370	*seabrana*	10119	BA	cf	6
44	CPI110370B	*seabrana*		BA		
14	CPI110370C	*seabrana*		BA		
8	CPI110372	*seabrana*	10517	BA	cf	6
78	CPI115993	*seabrana*	11578	BA		
40	CPI115994	*seabrana*	11583	BA		
77	CPI115995	*seabrana*	11585	BA		
32	CPI92476	*seabrana*	2107	BA	cf	6
74	CPI92838B	*seabrana*	cv. Primar[7]	MG		

[1] Member number is ID code used for data recording and pattern analysis.

[2] Origin collection numbers refer to CPI and ATF (CSIRO) accession numbers.

[3] Species = species names.

[4] CIAT refers to CIAT germplasm collection numbers.

[5] State abbreviations for Brazil: BA = Bahia, MG = Minas Gerais, ZUL = Zulia (Venezuela).

[6] Plant type and region refer to classification/code by Maass (1989): aff = 'aff.-scabra-type', bra = coastal Brazilian type, con = continental type, cf = 'cf.-scabra-type', po = selected from CIAT germplasm; Region = geographical distribution.

[7] Australian selected cultivars.

gated after planting to ensure establishment. The field site was located on a fertile alluvial soil and no fertiliser was applied during the trial. All *S. seabrana* accessions were inoculated with *Bradyrhizobium* strains CB3053 and CB3481, *S. scabra* with CB82 and *S. hamata* with CB1650 and CB2126.

Attributes

A data set of 12 nominal (N), 7 binary-presence/absence (B) and 20 ratio (R) attributes (Table 2) was recorded for each of the 79 lines of *Stylosanthes*. Habit, leaflet, bristle and hair attribute data were recorded in May 1997 by harvesting the fifth plant in each row and plant yield, inflorescence and seed data were obtained in June 1997 by harvesting 2 random plants per row.

Classification methods

The data set of accessions (rows) by attributes (columns) was analysed with PATN (Belbin 1995). The module DATN was used to convert the nominal and presence/absence data to ratio format, thus providing 52 attributes for the analysis. The module ASO, with the Gower Metric option, was used to obtain symmetric matrices, which were classified by the hierarchical routine FUSE (UPGMA option). The routine GDEF was used to determine group composition and GSTA to determine which attributes contributed most to the formation of the groups. Routines DEND (Dendrogram), MST (Minimum Spanning Tree) and PCA (Principal Components Analysis) were used to display group structure and relationships among groups. NNB (Nearest Neighbours) was used to assess proximity relationships and BOND to indicate the strength of the relationship between nearest neighbours. MASK was used to remove non-contributing data (Belbin 1995).

Results

Classification

At the 5-group level, the attributes lanceolate terminal leaflet shape, cylindrical/obovate seed shape and dark seed colour separated out *S. hamata* cv. Amiga as a single member group. The MASK module was used to remove these

Table 2. Morphological and agronomic attributes used in the classification.

Character attributes	Attribute type[1]	Observations (States)
Growth habit	N	1, erect; 2, semi-erect; 3, prostrate
Stem bristles	N	1, absent; 2, stipule and node; 3, all over
Stem hairs	N	1, absent; 2, one side of stem; 3, all over
Leaf bristles	N	1, absent; 2, around margin
Leaf hairs	N	1, absent; 2, back of leaflet; 3, all over
Terminal leaflet shape	N	1, lanceolate; 2, elliptical; 3, obovate
Terminal leaflet apex	N	1, acute; 2, obtuse
Beak length relative to upper articulation	N	0 > upper articulation; 1 < upper articulation
Pod pubescence	N	1, clean; 2, small bristles; 3, hairy
Pod beak curl	N	1, straight; 2, curled
Seed shape	N	1, cylindrical, oblong; 2, square; 3, rectangular
Seed colour	N	1, pale; 2, medium; 3, dark
Anthracnose resistance	B	0, not resistant; 1, resistant
Stem viscidity	B	0, not viscid; 1, viscid
Stipule horn terminal bristle	B	0, absent; 1, present
Stipule horn lateral bristles	B	0, absent; 1, present
Inflorescence bristles	B	0, absent; 1, present
Inflorescence viscidity	B	0, not viscid; 1, viscid
Mixed seed colour	B	0, no; 1, yes
Stem thickness 5 cm above ground	R	(mm)
Stem thickness 5 cm behind apices	R	(mm)
Stem length	R	(cm)
Total plant yield	R	(g)
Petiole length	R	(mm)
Terminal leaflet width	R	(mm)
Terminal leaflet length	R	(mm)
Ratio leaflet length:width	R	(%)
Beak length	R	(mm)
Pod length	R	(mm)
Seed yield/plant	R	(g)
Hardseededness	R	(%)
Seed weight (1000 seeds)	R	(g)

[1]N = nominal (converted to ratio); B = binary/presence/absence (converted to ratio); R = Ratio.

attributes and *S. hamata* from the data set, which was re-analysed using the same PATN modules (49 attributes and 78 lines). The resulting dendrogram and group membership at the 5-group level are shown in Figure 1, and the attributes in decreasing order of contribution to the classification in Table 3. Group membership is recorded in an MST (Figure 2) and PCA (Figure 3). Coordinates 1, 2 and 3 of the PCA accounted for 78% of the total variation: Coordinate 1 – 39%, Coordinate 2 – 25% and Coordinate 3 – 14%.

The separation between Groups 1–3 and Groups 4 and 5 is based on terminal leaflet shape (elliptical or obovate) and terminal leaflet apex shape (acute or obtuse), the presence or absence of leaflet and stem hairs, the presence or absence of inflorescence bristles and the presence or absence of stipule horn lateral bristles. Groups 1 and 2 are separated by the presence or absence of stem hairs and presence or absence of a stipule horn terminal bristle. Group 3 comprises only 2 members and is separated from, but closely allied to, Group 2 by the variable combination of leaf hair and stipule horn bristle attributes. The one member of Group 5 is separated from Group 4 by a different combination of the stem bristle and terminal leaflet characteristics.

Group descriptions

Group 1 contains 35 members, of which 33 are listed as *S. seabrana* and 2 members (31 and 39) as *S. scabra*. All members have narrowly elliptical to obovate (terminal) leaflets with long bristles on the margins and midribs, leaflets without hairs, and the characteristic stem hairs on only one side of the stem. This group corresponds to the cf.-scabra-types of Maass (1989).

Group 2 contains 25 members, of which 23 are listed as *S. scabra* (the aff.-*scabra*-types of Maass 1989) and 2 members (55 and 58) listed as *S. seabrana*. These members also have elliptical to obovate leaflets and long bristles on margins and midrib of leaflets but differ by having stem hairs occurring all over the stem and a stipule horn terminal bristle. Group 3 contains only 2

Table 3. Character attributes contributing most to the classification.

Character attribute	Relative Kruskal-Wallis value	Group 1[1]	Group 2[1]	Group 3[1,2]	Group 4[1]	Group 5[1,2]
Terminal leaflet shape, elliptical	1.00	Yes	Yes	Yes	Yes	No
Terminal leaflet shape, obovate	1.00	Yes	Yes	Yes	Yes	No
Terminal leaflet apex, acute	1.00	Yes	Yes	Yes	Yes	No
Terminal leaflet apex, obtuse	1.00	No	No	No	No	Yes
Leaflet hair, all over	0.92	No	No	No	Yes	Yes
Leaflet hair, absent	0.88	Yes	Yes	Yes/No	No	No
Stem hair, all over	0.86	No	No/y	Yes	Yes	Yes
Inflorescence bristles	0.81	Yes	Yes	Yes/No	No	No
Stem hair, one side	0.77	Yes/n	No/y	No	No	No
Inflorescence viscidity	0.68	Yes	Yes	No	Yes/n	Yes
Terminal leaflet width (mm)	0.65	5.3	3.5	3.4	6.7	4.9
Stipule horn terminal bristles	0.64	No/y	Yes/n	Yes/No	No/y	No
Stipule horn lateral bristle	0.62	Yes	Yes/n	Yes/No	No/y	No
Leaflet bristle, margin	0.57	Yes/n	Yes/n	Yes/No	No/y	No
Leaflet bristle, absent	0.57	No/y	No/y	Yes/No	Yes/n	Yes
Stem bristles, absent	0.56	No/y	No/y	Yes/No	Yes/n	Yes
Leaflet hair, back of leaflet	0.50	No	No	Yes/No	No	No
Stem bristles, stipule and node	0.47	Yes/n	Yes/No	No	No/y	No
Pod pubescence, hairy	0.47	Yes/No	No	No	Yes/n	Yes
Pod length (mm)	0.46	8.9 (0.9)[3]	7.5 (2.0)	8.1 (0.8)	7.3 (1.0)	9.6
Terminal leaflet length (mm)	0.44	14.4 (3.7)	9.8 (2.8)	10.1 (0.2)	16.1 (3.1)	6.2
Seed wgt (1000 seeds) (g)	0.44	0.2 (0.04)	0.2 (0.01)	0.2 (0.04)	0.2 (0.05)	0.2
Seed yield (g)	0.43	40.9 (21.3)	15.4 (12.4)	18.1 (17.1)	13.9 (16.6)	1.8
Plant yield (g/plant)	0.28	504 (198)	280 (140)	345 (71)	452 (303)	106

[1]Key: Yes = all members of group with this character attribute.
No = no members of group with this character attribute.
Yes/No = about equal number of members with/without this character attribute.
Yes/n = 1 or 2 members of group without this character attribute.
No/y = 1 or 2 members of group with this character attribute.

[2]Note Group 3 has only 2 members and Group 5 only 1 member.

[3]( ) standard deviation.

members with variable characteristics. They are most closely allied to members of Group 2. At 5 BOND levels (strongest = 5 to weakest = 1), these 2 members (64 and 73) were associated with Group 2 member (38) at level 2. The BOND between Group 2 members (38) and (12) by comparison was at level 5 (See Figure 2, MST). Some BOND strength information is indicated by a circled number (the bond level) in Figure 2.

Group 4 has 14 members, that include the 2 *S. scabra* cultivars and is characterised by the absence of leaflet bristles but with hairs all over the stem and leaflets. All members of the group are *S. scabra* and most have provenances of Minas Gerais in Brazil. Member (71) has similarities to both Groups 2 and 4 but BOND strengths are at the weakest levels. Group 5 has only a single member (35, *S. scabra*) and it is most closely allied to Group 4, differing in terminal leaflet apex shape and seed shape, but only at bond level 2 from member (5).

Agronomic plant yield, seed yield, stem length and hardseededness did not contribute significantly to the separation of members into the 5 groups. Mean values with standard deviation for terminal leaflet length, seed yield, seed weight per 1000 seeds and plant yield are included in Table 3.

Discussion

The classification separated the 79 accessions into 5 mostly homogenous groups representing *S. seabrana* (*S.* sp. cf-scabra-type) (Group 1), *S. scabra* (*S.* sp. aff.-scabra-type) (Group 2) and *S. scabra* (Group 4). There were several anomalies. The initial analysis, with all 52 attributes and 79 accessions of *Stylosanthes*, separated out *S. hamata* cv. Amiga as strongly different from the other 78 accessions, having dark coloured, cylindrical/oblong seeds and a lanceolate terminal leaflet. On this basis, *S. hamata* cv. Amiga and

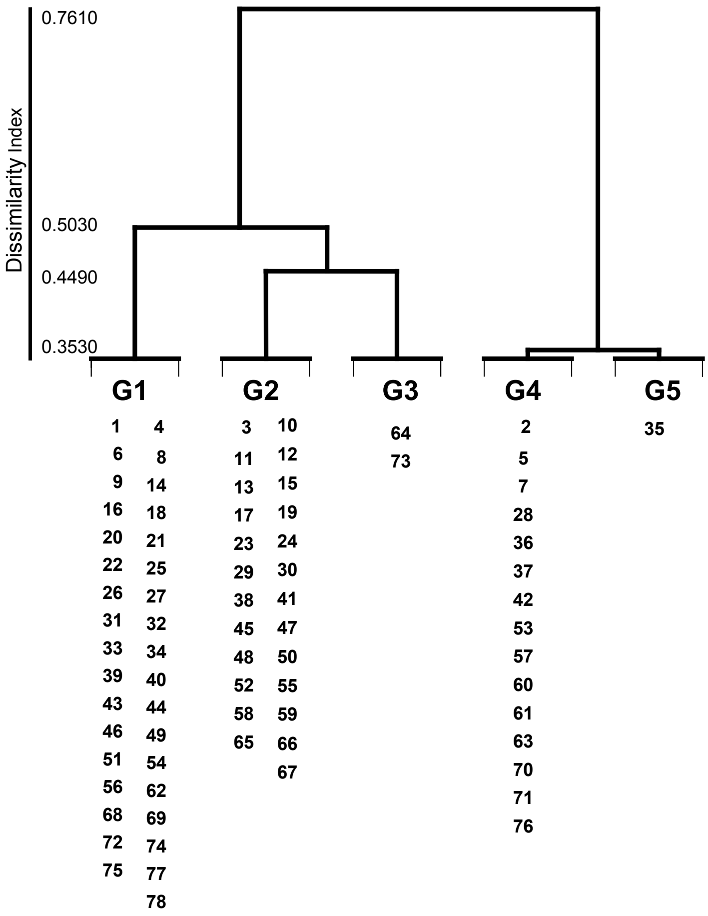

Figure 1. Group membership and dendrogram showing relationships between 78 accessions of *Stylosanthes* x 49 attributes – refer to Table 1 for identification of accessions in each group. Numbers on y-axis indicate dissimilarity levels between groups. G1–G5 refer to groups defined by PATN.

the attributes lanceolate terminal leaflet shape, cylindrical seed shape and dark seed colour were removed from the data set. Accession ATF2351 (35) (from north-west Bahia, Brazil) also is clearly different from the rest but is most similar to *S. hamata* cv. Amiga, having a beak length ≥ upper article and leaflets without bristles. Maass and Mannetje (2002) used these characteristics in the first couplet of their taxonomic key to distinguish between *S. hamata* and *S. seabrana*/*S. scabra*. In the absence of *S. hamata* data in the reanalysis, ATF2351 (35) still separated out as a single member for Group 5 and thus may be a hybrid type. Similarly, ATF2339 (64) and ATF2340 (73) are not similar to each other but have characteristics most like those of Group 2. The low level of their relatedness is obvious from the distances and bond strengths [see members (64) and (73) in Figure 2. MST].

Accessions ATF2521 (55) and ATF2533 (58) from Group 2 are identified in germplasm databanks as *S. seabrana* and ATF2335 (31), ATF2341 (39) and ATF2519 (43) from Group 1 and ATF2339 (64) and ATF2340 (73) from Group 3 are identified as *S. scabra*. This study suggests that all members of Group 1 should be identified as *S. seabrana.* Most of the Maass (1989) 'aff-scabra-types' in Group 2 could be recognised as a separate taxon, based on the presence of a long terminal bristle on the stipule horn. We

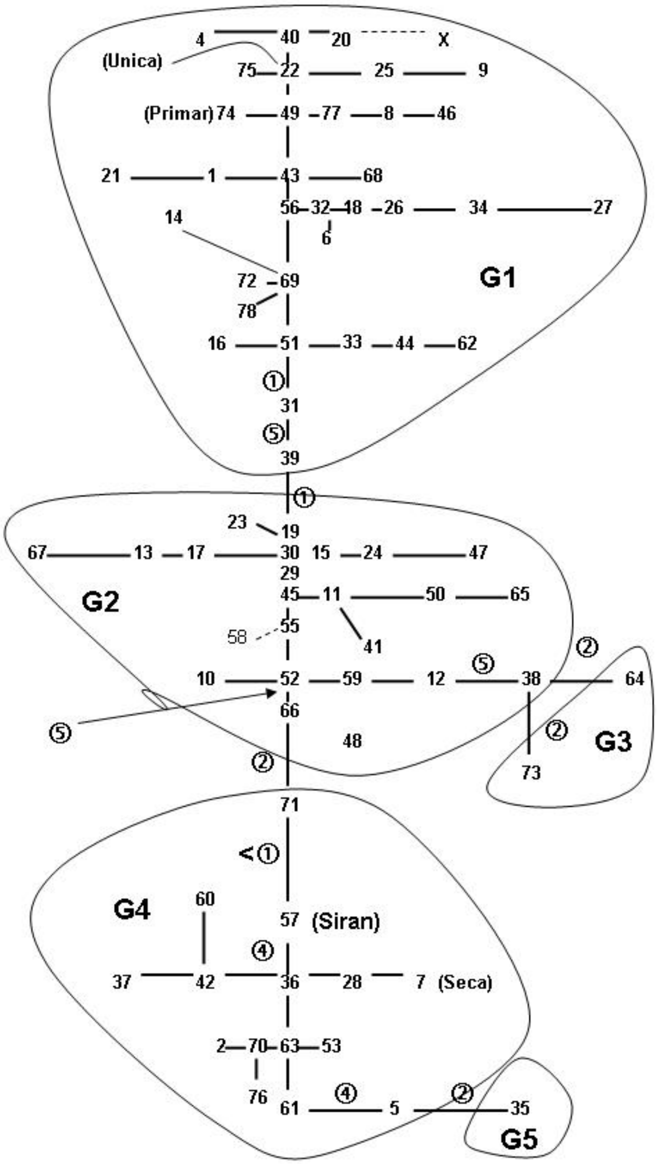

Figure 2. Topographical representation of the MST for distribution of accessions of *Stylosanthes* for morphological character attributes – refer Table 1 to identify accessions. Distance between accessions is a relative measure of closeness (or dissimilarity) between accessions. Numbers in circles indicate BOND strength.

refer to it as 'BM-aff.-type–scabra'. ATF2539B (71) is listed as *S. seabrana* and clearly should be listed as *S. scabra*. RAPD and STS genetic marker assessments (Liu 1997) also suggest that ATF2539B (71) is *S. scabra*. ATF2532 (60) is responding as a *S. scabra*. We believe that many of these suggested name changes are the result of mis-identifications at the time of seed collection. Some may be the result of accidental seed contamination, either at collection or during seed increase activities. *Bradyrhizobium* response data (Date 2010) support the suggestion that members (60) and (71) are *S. scabra*. Similarly, but in the reverse way, ATF2335 (31) and ATF2341 (39)

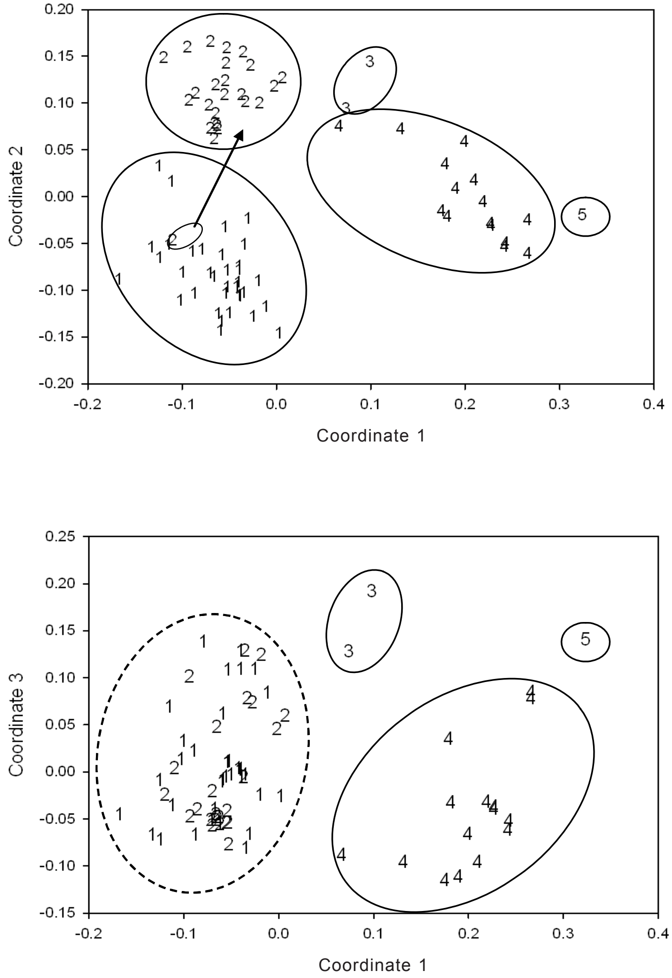

Figure 3. Plot of PCA coordinates 1 and 2 and of 1 and 3 showing major grouping. Numbers represent members in each of the 5 groups defined in Figure 1.

are listed as *S. scabra* but are grouped with the *S. seabrana* (Group 1) and again *Bradyrhizobium* response data suggest that these accessions are *S. seabrana* (Date 2010).

The 16 accessions of *S. seabrana* (*S.* sp. aff. *S. scabra*) used by Jansen and Edye (1996) were spread over 3 groups; however, when included within this new assessment, all 16 were located in Group 1 with 15 of the additional accessions. Of the 4 other accessions listed as *S. seabrana,* members (55) and (58) are located in closely allied Group 2. Member (71) clearly is not *S. seabrana* [maybe mixed seed with member (25) at collection, since ATF2539B (71) was identified from the original ATF2539 (25) collection]. There is some disparity between the classification of cultivars Siran and Seca between Jansen and Edye (1996) and this work. Both are cultivars of *S. scabra* and are located in the same group in this study but were well separated in the earlier work. Given that the same set of attributes was used in both studies, we attribute this difference to a lower level of dissimilarity in the more recent work.

Sixty-six of the new accessions in this study originated from the state of Bahia in Brazil.

The remaining 10 were from the neighbouring state of Minas Gerais. Most of those in Group 1 have provenances towards the northern half of this region, while those in Group 2 are from the southern half. Effectiveness of nitrogen fixation in response to strains of *Bradyrhizobium* also place these accessions into separate groups, Group 1 being those effectively nodulated by only a few strains of *Bradyrhizobium,* while those in Group 2 were effective with a range of strains (Date 2010). Such separation lends support to the suggestion of a separate ecotype or taxon for the Group 2 members referred to as 'BM-aff.-types'. The availability of effective (Date 2010) and field competent strains of *Bradyrhizobium* (R.A. Date unpublished data) for *S. seabrana* provide a basis for further agronomic evaluation of members of these 2 groups to select new legume cultivars adapted to a range of edaphic situations for both the cropping and pastoral industries.

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