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Bradyrhizobium effectiveness responses in *Stylosanthes hamata* and *S. seabrana*

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Abstract

A collection of *Stylosanthes hamata* and *S. seabrana* accessions were screened for effectiveness of nitrogen fixation with a range of strains of *Bradyrhizobium* and grouped using PATN Analysis package for likeness of their response patterns. The accessions divided into 2 major groups, 1 with broad spectrum response being effective with many strains of *Bradyrhizobium* and the other with a specific strain requirement, nodulating effectively with a narrow range of strains of *Bradyrhizobium.* Most promiscuously nodulating accessions have provenances from wetter environments with soil of pH less than 7, whereas the specific strain requiring groups were from seasonally drier environments with slightly alkaline soils. Similarly, most of the promiscuous groups were tetraploid and the specific groups diploid.

Introduction

Edye *et al*. (1984) were the first to discuss the importance of climate and edaphic adaptation as a basis for selection of new cultivars of the pasture legume *Stylosanthes* for northern Australian conditions. Detailed studies of agronomic variation in a wide range of *Stylosanthes* spp. to assess their suitability as pasture legumes for this environment had begun in the 1960s, when only *S. guianensis* and *S. humilis* were recognised as pasture legumes. By 1984, 13 cultivars from 5 species had been released (Edye and Cameron 1984). Most of these cultivars had been selected for their adaptation, persistence and dry matter production, including nitrogen fixation, in infertile soils of the semi-arid and seasonally dry tropical environments of northern Australia. None of these cultivars required inoculation with special strains of *Bradyrhizobium* and all nodulated effectively with established soil strains of bradyrhizobia (Date 1984; 1991). However, as evaluation programs became more selective and targeted towards finding new cultivars for special conditions, particularly in the heavier clay soils of the cropping regions, many accessions failed to nodulate with existing bradyrhizobia and required inoculation with selected strains for successful establishment and growth (R.A. Date and L.A. Edye unpublished data). Special regional adaptation conditions included tolerance to cold, infertile low pH soils with high soluble aluminium content, and neutral to alkaline clay soils in cropping areas.

An integral part of CSIRO's selection program was the screening of potentially useful accessions of *Stylosanthes* for their effectiveness in nitrogen fixation with a range of strains of *Bradyrhizobium*. Significant differences between and within species for the specificity of bradyrhizobial strains to fix nitrogen had been reported for some accessions of *S. guianensis* and *S. hamata* (Date and Norris 1979). More recently, specificity for both ability to form nodules and to effectively fix nitrogen have been reported in *S. capitata* (Date 1984), *S. seabrana* (as *S*. sp. aff. *S. scabra*) (Date *et al*. 1996) and *S. macrocephala* (Date and Eagles 2010).

This paper summarises the *Bradyrhizobium* screening experiments associated with the cultivar selection program for *S. hamata* and *S. seabrana*. The screening aimed to assess relative responses in nitrogen-fixation effectiveness to a group of strains of bradyrhizobia and assess any variability in this response between the accessions of *Stylosanthes*. The screening began in 1981 as the agronomic selection program targeted *S. hamata* and *S. scabra* accessions, mostly from Brazil and Venezuela, for their adaptability to neutral to alkaline clay soils of the cropping

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regions of Queensland. As evaluation programs continued in the late 1980s, a set of accessions adapted to these conditions was identified. These accessions correspond to a morphologically and agronomically different set of germplasm first described by Maass (1989) as *S. scabra* 'cf.-scabra-type' and later assessed for a wider range of morphological and agronomic attributes by Jansen and Edye (1996). Unfortunately, Jansen and Edye (1996), Date *et al*. (1996) and Liu and Musial (1997) referred to these accessions as *S.* sp.aff. *S. scabra*. More recently, this same material has been described as a new species, namely *S. seabrana* (Maass and Mannetje 2002). Selection of suitable strains of *Bradyrhizobium* for this new species and for the diploid *S. hamata* germplasm collected originally in Venezuela required special germplasm collection trips to Venezuela and Brazil as no suitable strains existed in the then CSIRO Rhizobium Germplasm Collection (R.A. Date unpublished data). Similarly, no suitable strains were available from the International Centre for Tropical Agriculture in Colombia (CIAT) or the research institute EMBRAPA of Brazil.

Materials and methods

Germplasm

In the period 1981–1996, 21 separate experiments were completed in which 166 entries of *S. hamata*, 4 of *S. scabra*, 29 of *S*. sp. aff. *S. scabra* (labeled affB in Table 1), 2 of *S*. sp. aff. *S. hamata* (labeled affC in Table 1), 36 of *S. seabrana* (previously *S*. sp. aff. *S. scabra* of Date *et al.* 1996 and 8 of which were the 'cf. scabra-type' of Maass 1989), 2 of *S. calcicola*, 3 of *S. humilis*, 3 of *S. sympodialis* and 1 of *S*. sp. (Table 1) were screened against 20 strains of bradyrhizobia. The accessions of *S. calcicola, S. humilis* and *S. sympodialis* were included as additional potentially useful plants based on regional evaluation trials (L.A. Edye and R.J. Williams personal communication). One entry of *S. hamata* cv. Verano (T = tetraploid) and one of *S. hamata* CPI40264A (D = diploid) were included in each experiment as control standards. A total of 295 entries (including the 2 standards in each experiment as separate entries, and 9 repeats) were used to assess responses to strains of *Bradyrhizobium*.

The first 4 experiments (1981–1982) compared 76 entries against 12 strains of *Bradyrhizobium*, then 15 experiments (1987–1992) compared 160 entries against 18 strains and 2 experiments (1995–1996) compared 59 entries against 11 strains. The range of strains was expanded in the 1987–1992 experiments to include more strains effective with the diploid sets of *S. hamata* accessions. The range of strains used in the 1995–1996 experiments was necessary to ensure that there were enough strains effective with *S. seabrana*. The 1981–1982, 1987–1992 and 1995–1996 experiments are referred to throughout as Ha76, Ha160 and Ha59, respectively. The composite experiments of 295 accessions x 6 strains (common to all entries) and 295 accessions x 20 strains (some missing values), similarly, are referred to as Ha295 and HaAll.

Table 2 lists the strains of *Bradyrhizobium* used in the screening experiments, their host of origin and provenance. Reasons for selection and changes in test strains are detailed in the Discussion.

Plant growth conditions and measurements

Pre-germinated seeds of each accession were sown aseptically into a nitrogen-free system (Norris and Date 1979). Duplicate sand-jars of each accession x strain combination were inoculated with *Bradyrhizobium* 5–7 days after sowing. Uninoculated and nitrogen controls were included. Nitrogen was added to the nitrogen controls as a 5% solution of KNO_3 at a rate equivalent to 30 kg/ha N. The plants for each experiment were maintained in a glasshouse during either March-May or October-November in Brisbane with air and 'soil' temperatures between 20 and 30^{o}C. Plants were harvested after 6–8 weeks, when roots were washed free of sand and nodulation recorded as '—' (none), '+' (few) or '++' (many). The dry weights (g) of whole plants (4/sand-jar) were used as an index of the effectiveness of nitrogen fixation.

Data analysis

Plant dry weight values were standardised as a percentage of the value for the relevant nitrogen control. The pattern analysis program PATN (Belbin 1995) was used to group like response patterns to a limited number of groups. The module ASO, with the Gower Metric option, was used to obtain symmetric matrices, which

Table 1. Source of accessions of *Stylosanthes hamata*, *S. scabra*, *S. seabrana* and *S.* spp., their CPI/ATF identification number, ploidy level and serial number for screening with strains of *Bradyrhizobium* for effectiveness of nitrogen-fixing associations.

CPI[1]	Sp[2]	Pl[3]	Serial[4]	Gp[5]	Location	S/P[6]	Ctry[6]	Lat.[6]	Long.[6]	Rain[6]	Eln[6]	pH[7]
55821	ham	T	84	6	38 km Maracaibo-San Rafael	ZUL	VEN	10.58N	71.45W	480	30	
65962	ham	T	246	6	Bonda	MAG	COL	11.17N	74.05W	600	50	
65965	ham	T	20	6	17 km Santa Marta-Minca	MAG	COL	11.18N	74.12W	1000	500	6.2
105678	ham	(D)	153	6	49 kmE Brumado, 39 kmW Anaje, BR262	BA	BRA	14.39S	41.25W			
105678	ham	(D)	183	6	49 kmE Brumado, 39 kmW Anaje, BR262	BA	BRA	14.39S	41.25W			
109326	ham	T	62	6	13 kmE Namatoco-Riohacha	MAG	COL	11.15N	74.03W	350	340	6.0
109344	ham	T	73	6	17 kmE Palomino-Riohacha	GUA	COL	11.13N	73.24W	2220	10	7.2
110024	ham	T	1	6	Maracaibo, 2 km Cairzu Lab.	ZUL	VEN	10.34N	71.44W	490	66	7.5
110025	ham	T	2	6	Maracaibo, Internat. Airport	ZUL	VEN	10.34N	71.44W	490	66	7.5
110026	ham	T	3	6	Maracaibo, Bot. Gdns, Palito Blanco Rd	ZUL	VEN	10.34N	71.44W	490	66	7.5
110027	ham	T	4	6	Maracaibo, Km18, Palito Blanco Rd	ZUL	VEN	10.34N	71.44W	490	66	7.5
110029	ham	T	6	6	Maracaibo, Km18, Palito Blanco Rd	ZUL	VEN	10.34N	71.44W	490	66	7.5
110029	ham	T	78	6	Maracaibo, Km18, Palito Blanco Rd	ZUL	VEN	10.34N	71.44W	490	66	7.5
110030	ham	T	7	6	Maracaibo, Cairzu Lab.	ZUL	VEN	10.34N	71.44W	490	66	7.5
110037	ham	T	13	6	14 kmNE El Carreral-Moina	ZUL	VEN	11.16N	72.07W	716	50	7.0
110039	ham	T	15	6	Maracaibo, near Maruma Hotel	ZUL	VEN	10.34N	71.44W	490	66	7.5
110040	ham	T	16	6	Maracaibo, near Maruma Hotel	ZUL	VEN	10.34N	71.44W	490	66	7.5
110041	ham	T	43	6	Maracaibo, near Maruma Hotel	ZUL	VEN	10.34N	71.44W	490	66	7.5
110043	ham	T	80	6	Maracaibo, near Maruma Hotel	ZUL	VEN	10.34N	71.44W	490	66	7.5
110095	ham	T	101	6	Carora Airport		VEN	10.11N	70.05W	618	411	8.0
110109	ham	T	120	6	Km19, 14 kmN Quibor-Barquisimeto	LAR	VEN	10.00N	69.32W	410	600	7.0
110116	ham	T	45	6	Las Dantas, 2 kmS Peracal-Rubio	TAC	VEN	07.47N	72.26W	1189	867	6.2
110134	ham	T	122	6	Cementerio La Chinita, Maracaibo	ZUL	VEN	10.34N	71.44W	490	66	7.5
110135	ham	T	123	6	Cementerio La Chinita, Maracaibo	ZUL	VEN	10.34N	71.44W	490	66	7.5
110138	ham	T	124	6	Botanic Gardens, Maracaibo	ZUL	VEN	10.34N	71.44W	490	66	7.5
110316	ham	T	71	6	Tigreia, near Minca	MAG	COL	11.10N	74.07W	380	225	6.0
55822C	ham	T	127	6	12 km Maracaibo-San Rafael	ZUL	VEN	10.48N	71.40W	460	30	
55822D	ham	T	128	6	12 km Maracaibo-San Rafael	ZUL	VEN	10.49N	71.41W	460	30	
55822E	ham	T	129	6	12 km Maracaibo-San Rafael	ZUL	VEN	10.50N	71.42W	460	30	
55822F	ham	T	130	6	12 km Maracaibo-San Rafael	ZUL	VEN	10.51N	71.43W	460	30	
55822G	ham	T	131	6	12 km Maracaibo-San Rafael	ZUL	VEN	10.52N	71.44W	460	30	
55822A	ham	T	85	6	12 km Maracaibo-San Rafael	ZUL	VEN	10.48N	71.40W	460	30	
55822B	ham	T	86	6	12 km Maracaibo-San Rafael	ZUL	VEN	10.49N	71.41W	460	30	
VERANO	ham	T	8	6	Maracaibo Airport	ZUL	VEN	10.36N	71.42W		30	
VERANO	ham	T	17	6	Maracaibo Airport	ZUL	VEN	10.36N	71.42W		30	
VERANO	ham	T	30	6	Maracaibo Airport	ZUL	VEN	10.36N	71.42W		30	
VERANO	ham	T	40	6	Maracaibo Airport	ZUL	VEN	10.36N	71.42W		30	
VERANO	ham	T	52	6	Maracaibo Airport	ZUL	VEN	10.36N	71.42W		30	
VERANO	ham	T	63	6	Maracaibo Airport	ZUL	VEN	10.36N	71.42W		30	
VERANO	ham	T	82	6	Maracaibo Airport	ZUL	VEN	10.36N	71.42W		30	
VERANO	ham	T	96	6	Maracaibo Airport	ZUL	VEN	10.36N	71.42W		30	
VERANO	ham	T	104	6	Maracaibo Airport	ZUL	VEN	10.36N	71.42W		30	
VERANO	ham	T	118	6	Maracaibo Airport	ZUL	VEN	10.36N	71.42W		30	

CPI[1]	Sp[2]	Pl[3]	Serial[4]	Gp[5]	Location	S/P[6]	Ctry[6]	Lat.[6]	Long.[6]	Rain[6]	Eln[6]	pH[7]
VERANO	ham	T	125	6	Maracaibo Airport	ZUL	VEN	10.36N	71.42W		30	
VERANO	ham	T	138	6	Maracaibo Airport	ZUL	VEN	10.36N	71.42W		30	
VERANO	ham	T	149	6	Maracaibo Airport	ZUL	VEN	10.36N	71.42W		30	
VERANO	ham	T	159	6	Maracaibo Airport	ZUL	VEN	10.36N	71.42W		30	
VERANO	ham	T	184	6	Maracaibo Airport	ZUL	VEN	10.36N	71.42W		30	
VERANO	ham	T	216	6	Maracaibo Airport	ZUL	VEN	10.36N	71.42W		30	
VERANO	ham	T	218	6	Maracaibo Airport	ZUL	VEN	10.36N	71.42W		30	
VERANO	ham	T	238	6	Maracaibo Airport	ZUL	VEN	10.36N	71.42W		30	
VERANO	ham	T	259	6	Maracaibo Airport	ZUL	VEN	10.36N	71.42W		30	
VERANO	ham	T	280	6	Maracaibo Airport	ZUL	VEN	10.36N	71.42W		30	
VERANO	ham	T	294	6	Maracaibo Airport	ZUL	VEN	10.36N	71.42W		30	
70522	ham.affC	(D)	253	6	Riviera Beach	FL	USA	26.46N	80.06W			
70529	ham.affC	(D)	256	6	Juno	TX	USA	30.11N	101.09W			
61667	hum	(X)	284	6			VEN					
61674	hum	(X)	285	6			VEN					
68841	hum	(X)	286	6	95 km Palomino-Riohacha	GUA	COL	11.30N	73.00W			5.4
55803	sca	(X)	292	6	76 km Feira de Sanatana-Jequie	BA	BRA	12.30S	39.32W	550	200	
55805	sca	(X)	293	6	100 km Itaberaba-Seabra	BA	BRA	12.32S	41.23W	600	500	
ATF2328	sca.affB	(T)	168	6		BA	BRA	12.24S	41.52W	780	1150	
ATF2329	sca.affB	(T)	200	6		BA	BRA	12.27S	41.39W	830	1050	
ATF2330	sca.affB	(T)	169	6		MG	BRA	15.20S	43.45W	930	490	
ATF2331	sca.affB	(T)	170	6		BA	BRA	14.49S	39.17W	1920	60	
ATF2332	sca.affB	(T)	171	6		BA	BRA	14.49S	39.11W	2010	50	
ATF2333	sca.affB	(T)	201	6		BA	BRA	14.56S	39.19W	1900	80	
ATF2334	sca.affB	(T)	202	6		BA	BRA	15.14S	39.22W	2000	130	
ATF2335	sca.affB	(T)	203	6		BA	BRA	13.53S	39.26W	1100	150	
ATF2337	sca.affB	(T)	205	6		BA	BRA	14.49S	39.11W			
ATF2338	sca.affB	(D)	172	6		BA	BRA	12.58S	41.51W	900	1140	
ATF2339	sca.affB	(T)	206	6		BA	BRA	14.10S	39.30W	1130	110	
ATF2341	sca.affB	(T)	173	6		BA	BRA	14.40S	40.27W	510	890	
ATF2342	sca.affB	(T)	174	6		BA	BRA	15.16S	41.06W	840	850	
ATF2343	sca.affB	(T)	175	6		MG	BRA	15.53S	41.24W	1000	950	
ATF2344	sca.affB	(T)	207	6		MG	BRA	16.24S	41.32W	760	610	
ATF2345	sca.affB	(T)	176	6		MG	BRA	17.08S	41.32W	880	540	
ATF2346	sca.affB	(T)	208	6		MG	BRA	17.18S	41.31W	980	650	
ATF2347	sca.affB	(T)	179	6		MG	BRA	17.54S	41.25W	980	970	
ATF2348	sca.affB	(T)	209	6		MG	BRA	18.28S	41.50W	1250	230	
ATF2349	sca.affB	(T)	178	6		BA	BRA	13.38S	41.21W	650	500	
ATF2352	sca.affB	(T)	210	6		MG	BRA	18.46S	41.59W	1250	300	5.5
ATF2353	sca.affB	(T)	211	6		MG	BRA	18.46S	41.59W	1250	300	
ATF2354	sca.affB	(T)	212	6		BA	BRA	12.30S	41.22W			
ATF2355	sca.affB	(T)	213	6		BA	BRA	14.34S	40.25W			
ATF2356	sca.affB	(T)	214	6		BA	BRA	14.40S	40.27W			
ATF2357	sca.affB	(T)	215	6		BA	BRA	12.26S	40.35W			
ATF2351	sca.sffB	(T)	180	6		BA	BRA	11.08S	45.12W	1000	670	

92476	sea	(D)	182	6	106 kmW Itaberaba	BA	BRA	12.30S	41.17W	850	520	
115995	sea	(D)	158	6	1 kmNW Lencois	BA	BRA	12.34S	41.23W	1190	400	
ATF2521	sea	(D)	191	6	25.2 kmN Utinga-Morro Do Chapeu	BA	BRA	11.55S	41.13W			
ATF2539B	sea	(T)	198	6	1 kmS BR242-Palmeiras	BA	BRA	12.30S	41.38W			
65958	sym	(X)	287	6	30 kmE Salinas-Guayaquil	GUA	ECU	02.15S	80.42W	500	100	7.1
65960	sym	X	142	6	Outside walled cemetery, Ancon	GUA	ECU	02.20S	80.47W	250	10	
67704B	sym	(X)	288	6	3 kmNW Playas-Progresso	GUA	ECU	02.37S	80.24W	500	20	6.3
65365	ham	T	19	5	Luz, Maracaibo	ZUL	VEN	10.34N	71.44W	490	66	7.5
65368	ham	T	244	5	Maracaibo	ZUL	VEN	10.44N	71.37W	550	10	
65371	ham	T	245	5	Calabozo	GUA	VEN	08.58N	67.28W			
68837	ham	T	21	5	95 km Palomino-Riohacha	GUA	COL	11.30N	73.00W			5.4
68838	ham	T	22	5	Santa Marta-Rodadero	MAG	COL	11.17N	74.11W			
68840	ham	T	23	5	Seashore near Santa Marta Airport	MAG	COL	11.13N	74.10W			
94444	ham	T	29	5			USA					
109320	ham	T	61	5	8 kmE Santa Marta-Riohacha	MAG	COL	11.15N	74.06W	310	70	7.0
109325	ham	T	33	5	3 kmNW Minca-Namatoco	MAG	COL	11.08N	74.05W	400	440	6.4
109331	ham	T	108	5	55 kmE Guachaca-Riohacha	MAG	COL	11.16N	73.48W	2220	10	6.5
109332	ham	T	72	5	8 kmE Guachaca-Riohacha	MAG	COL	11.15N	73.47W	2220	10	6.0
109347	ham	T	75	5	6 kmS Fonseca-Valledupar	GUA	COL	10.52N	72.54W	710	160	7.1
109349	ham	T	76	5	Valledupar Airport	MAG	COL	10.26N	73.14W	1200	200	6.3
109350	ham	T	77	5	14 kmSW Valencia De Jesus-Bosconia	MAG	COL	10.13N	73.28W	1480	135	5.7
110033	ham	T	10	5	114 kmSW Maracaibo-Machiques	ZUL	VEN	10.12N	72.26W	1400	100	7.5
110035	ham	T	11	5	La Cienega, 35 kmSW Maracaibo	ZUL	VEN	10.28N	71.54W	612	72	7.5
110036	ham	T	12	5	El Tigre, 9 kmSW El Carreral	ZUL	VEN	11.10N	72.14W	881	19	7.0
110038	ham	T	14	5	La Nueva Lucha, 25 kmNW Maracaibo	ZUL	VEN	10.48N	71.41W	564	2	7.5
110042	ham	T	79	5	Maracaibo, near Maruma Hotel	ZUL	VEN	10.34N	71.44W	490	66	7.5
110044	ham	T	35	5	Maracaibo, near Maruma Hotel	ZUL	VEN	10.34N	71.44W	490	66	7.5
110044	ham	T	81	5	Maracaibo, near Maruma Hotel	ZUL	VEN	10.34N	71.44W	490	66	7.5
110045	ham	T	87	5	Maracaibo, near Maruma Hotel	ZUL	VEN	10.34N	71.44W	490	66	7.5
110046	ham	T	132	5	68 kmS Maracaibo–Potrerito (LR4)	ZUL	VEN	10.22N	71.46W	608	3	7.5
110048	ham	T	88	5	37 kmS Maracaibo–Potrerito (LR4)	ZUL	VEN	10.27N	71.45W	608	3	7.5
110049	ham	T	89	5	24 kmSE Maracaibo, Bridge Toll Gate	ZUL	VEN	10.27N	71.23W	608	3	7.5
110050	ham	T	90	5	El Guanabano	ZUL	VEN	10.33N	71.14W	1288	120	7.0
110051	ham	T	91	5	38 kmSE Maracaibo-Carora (H17)	ZUL	VEN	10.29N	71.25W	600	100	7.0
110057	ham	T	36	5	Maracaibo, football stad. old airport	ZUL	VEN	10.34N	71.44W	490	66	7.5
110069	ham	T	95	5	Maracaibo, Paseo Del Lago	ZUL	VEN	10.34N	71.44W	490	66	7.5
110070	ham	T	98	5	Maracaibo, Ave 5th July	ZUL	VEN	10.34N	71.44W	490	66	7.5
110098	ham	T	39	5	Varadero	MAT	CUB	23.10N	81.16W			
110098	ham	T	102	5	Km487, 45 kmS Carora-Agua Viva	LAR	VEN	09.51N	70.11W	1048	560	6.5
110104	ham	T	110	5	1 kmN Sabana De Mendoza-Carora	TRU	VEN	09.26N	70.46W	1081	118	6.5
110162	ham	T	133	5	5 kmNE Altagracia-Quisiro	ZUL	VEN	10.44N	71.28W	504	30	7.
110166	ham	T	65	5	Maracay, Tapa Tapa-Palo Negro (H1)	ARA	VEN	10.15N	67.39W	881	436	6.5
110168	ham	T	66	5	Near El Parque, 24 kmE Nirgua	YAR	VEN	10.08N	68.25W	872	780	6.0
110205	ham	T	68	5	11 kmE Las Tejerias-Caracas (H1)	MIR	VEN	10.17N	67.05W	748	626	6.5
110206	ham	T	69	5	3 kmN Caracas Toll Gate-Maiquetia	DF	VEN	10.31N	66.54W	837	1035	7.0
110209	ham	T	70	5	37 kmE Maracay(Tapa Tapa)-Caracas	ARA	VEN	10.13N	67.21W	1002	625	6.5
110317	ham	T	147	5	Maracaibo	ZUL	VEN	10.34N	71.44W	490	66	7.5

CPI[1]	Sp[2]	Pl[3]	Serial[4]	Gp[5]	Location	S/P[6]	Ctry[6]	Lat.[6]	Long.[6]	Rain[6]	Eln[6]	pH[7]
CLON-4	ham	T	51	5			AUST	S	E			
ATF2336	sca.affB	(T)	204	4		BA	BRA	N	W			
61204	(sp.)	D	106	1	45 km Maicao-Valledupar	GUA	COL	11.18N	72.22W		150	
73524	cal	(X)	289	1	130 km Merida-Valladolid	YUC	MEX	20.38N	88.19W	1150	50	
73525	cal	(X)	290	1	288 km Merida-Puerto Juarez	QR	MEX	21.03N	87.01W	1450	10	
33205	ham	(D)	220	1	Port Louis	GT	GLP	16.25N	61.32W	1000	30	
33231	ham	(D)	221	1	Isla Verde	SJ	PRI	18.29N	66.08W		5	
36046	ham	(D)	222	1	Near Big Pine Key	FL	USA	24.41N	81.21W			
37038	ham	D	140	1	Santiago		DOM	19.30N	70.42W			
37038	ham	D	224	1	Santiago		DOM	19.30N	70.42W			
50997	ham	D	227	1			VEN		W			
50998	ham	D	228	1			VEN		W			
51391	ham	(D)	229	1		AND	BHS	24.45N	78.00W			
55826	ham	T	231	1	Maracaibo University	ZUL	VEN	10.44N	71.37W	460	30	
61670	ham	D	141	1			VEN		W			
62162	ham	D	241	1	Porlamar	NE	VEN	11.01N	63.54W			
65363	ham	D	242	1	Barranquilla	ATL	COL	11.10N	74.50W	850	100	
65364	ham	D	243	1	Barranquilla	ATL	COL	11.10N	74.50W	850	100	
70360	ham	(D)	247	1	Bendals	STJ	ATG	17.04N	61.50W			6.5
70366	ham	(D)	248	1	Coolidge	STG	ATG	17.08N	61.47W			7.9
70370	ham	(D)	249	1	Railway Line at Sugar factory	STG	ATG	17.08N	61.48W			
70371	ham	(D)	250	1	Road to highland		ATG	17.43N	61.38W			
70372	ham	(D)	251	1	Fattening area		ATG	17.43N	61.39W			
70374	ham	(D)	252	1	Highland		ATG	17.43N	61.37W			
70523	ham	(D)	254	1	Jupiter	FL	USA	26.57N	80.08W			
70525	ham	D	24	1	Florida City	FL	USA	25.27N	80.30W			
72850	ham	D	257	1	Boynton Beach	FL	USA	26.32N	80.04W			
72852	ham	D	258	1	Boca Raton	FL	USA	26.22N	80.04W			
72854	ham	D	261	1	Coral Gables	FL	USA	25.40N	80.17W			
72859	ham	D	25	1	Delray Beach	FL	USA	26.29N	80.04W			
73484	ham	(D)	262	1	Between Runways, Coolidge Airport	STG	ATG	17.08N	61.47W			7.9
73486	ham	(D)	263	1	Coolidge Airport	STG	ATG	17.08N	61.47W			
73487	ham	(D)	264	1	Coolidge Airport	STG	ATG	17.08N	61.47W			7.9
73488	ham	(D)	265	1	West Indies Oil	STJ	ATG	17.08N	61.50W			8.1
73491	ham	(D)	266	1	Shell Beach	STG	ATG	17.08N	61.46W			
73497	ham	(D)	267	1	Old Road, St Kitts		KNA	17.20N	62.48W			
73498	ham	(D)	268	1	Liberta Spa	ATG	17.02N	61.47W				
73499	ham	(D)	269	1	Shirley Heights	SPA	ATG	17.00N	61.45W			
73501	ham	(D)	270	1	Road to Fitches Creek	STC	ATG	17.06N	61.48W			
73505	ham	(D)	271	1	Sandy river area		ATG	17.40N	61.39W			
73506	ham	(D)	272	1		CUR	ANT	N	W			
73507	ham	(D)	273	1		CUR	ANT	N	W			
73509	ham	(D)	274	1		CUR	ANT	N	W			
73511	ham	D	26	1	Varadero	MAT	CUB	23.10N	81.16W			
73511	ham	D	275	1	Varadero	MAT	CUB	23.10N	81.16W			

73513	ham	(D)	276	1	Indian Castle, Nevis		KNA	17.07N	62.35W			
73514	ham	(D)	277	1	Round Hill, Nevis		KNA	N	W			
73515	ham	(D)	278	1	Indian Castle, Nevis		KNA	17.07N	62.35W			
73519	ham	(D)	282	1	Pinneys Beach, Nevis		KNA	17.09N	62.37W			
73523	ham	(D)	283	1	100 m from sea, Flamboyant Hotel	CUR	ANT	N	W			
94130	ham	D	27	1			USA	N	W			
94443	ham	D	28	1			USA	N	W			
99670	ham	D	54	1	Cocoa Beach	FL	USA	28.19N	80.36W			
109305	ham	D	55	1	14 kmW Barranquilla-Pto Colombia	ATL	COL	11.01N	74.54W	590	10	6.9
109307	ham	D	56	1	16 kmW Barranquilla-Pto Colombia	ATL	COL	10.59N	74.55W	560	10	7.2
109310	ham	D	57	1	10 kmE Salgar–Barranquilla	ATL	COL	11.01N	74.49W	630	10	7.4
109312	ham	D	58	1	Barranquilla, El Prado, Hotel El Golf	ATL	COL	10.58N	74.47W	730	40	6.9
109314	ham	D	59	1	Barranquilla, Bocas de Ceniza	ATL	COL	11.04N	74.49W	570	10	7.1
109316	ham	D	60	1	12 km Puente Pumarejo Santa Marta	MAG	COL	10.59N	74.41W	730	10	7.1
109346	ham	D	74	1	1 kmS Barrancas-Valledupar	GUA	COL	10.56N	72.48W	1580	140	7.2
110028	ham	T	5	1	Maracaibo, Palito Blanco Rd	ZUL	VEN	10.34N	71.44W	490	66	7.5
110066	ham	D	92	1	Maracaibo, near Graduate Ag. School	ZUL	VEN	10.34N	71.44W	490	66	7.5
110077	ham	D	109	1	Km153, 9 kmNW Marcillal-Coro	FAL	VEN	11.14N	68.54W	1100	130	8.5
110083	ham	D	99	1	Moruy	FAL	VEN	11.50N	69.59W	660	70	8.0
110087	ham	D	100	1	Km277, 20 kmSW Coro–Maracaibo	FAL	VEN	11.21N	69.51W	390	20	8.0
110108	ham	D	44	1	Km57, 8 kmN El Tocuyo-Barquisimeto	LAR	VEN	09.51N	69.45W	480	690	7.0
110110	ham	D	121	1	1 kmNE Agua Fria-San Filipe (LR3)	YAR	VEN	10.26N	69.00W	1200	360	7.5
110114	ham	D	111	1	Bella Vista Hotel, Porlamar, Margarita	NE	VEN	10.57N	63.51W	450	10	7.0
110119	ham	D	112	1	Castillo San Antonio, Cumana	SUC	VEN	10.26N	64.12W	355	100	7.5
110125	ham	D	113	1	10 kmNE Ocumare la Costa-Pto.Cata	ARA	VEN	10.29N	67.46W	824	35	7.5
110171	ham	D	114	1	Barquisimeto	LAR	VEN	10.14N	69.19W	516	613	7.5
110173	ham	D	134	1	Duaca turnoff, LR3	LAR	VEN	10.17N	69.10W	704	750	7.5
110174	ham	D	115	1	3 kmSW Licua-Barquisimeto (LR3)	LAR	VEN	10.19N	69.08W	704	750	7.5
110176	ham	D	135	1	4 kmSW Quibor-El Tocuyo (H7)	LAR	VEN	09.54N	69.39W	477	682	7.0
110181	ham	D	136	1	9kmNE Chejende-La Cuchilla	TRU	VEN	09.39N	70.19W	1094	1050	6.2
110185	ham	D	137	1	9 kmSW El Molino-Humocaro Bajo	LAR	VEN	09.43N	69.52W	490	693	6.8
110186	ham	D	144	1	8 kmN Humocaro Alto-El Molino (LR5)	LAR	VEN	09.39N	69.58W	753	950	6.8
110190	ham	D	116	1	San Juan de Lagunillas (H7)	MER	VEN	08.31N	71.21W	542	1100	7.0
110207	ham	D	117	1	7 kmN Caracas Toll Gate-Maiquetia	DF	VEN	10.31N	66.54W	873	835	7.0
110311	ham	D	146	1	Barranquilla	ATL	COL	11.10N	74.50W	730	40	7.4
40264A	ham	D	9	1		PE	BRA		W			
40264A	ham	D	18	1		PE	BRA		W			
40264A	ham	D	31	1		PE	BRA		W			
40264A	ham	D	41	1		PE	BRA		W			
40264A	ham	D	64	1		PE	BRA		W			
40264A	ham	D	83	1		PE	BRA		W			
40264A	ham	D	97	1		PE	BRA		W			
40264A	ham	D	105	1		PE	BRA		W			
40264A	ham	D	119	1		PE	BRA		W			
40264A	ham	D	126	1		PE	BRA		W			
40264A	ham	D	139	1		PE	BRA		W			
40264A	ham	D	150	1		PE	BRA		W			

CPI[1]	Sp[2]	Pl[3]	Serial[4]	Gp[5]	Location	S/P[6]	Ctry[6]	Lat.[6]	Long.[6]	Rain[6]	Eln[6]	pH[7]
40264A	ham	D	160	1		PE	BRA		W			
40264A	ham	D	260	1		PE	BRA		W			
40264A	ham	D	281	1		PE	BRA		W			
40264A	ham	D	295	1		PE	BRA		W			
61623A	ham	D	233	1	Near airport, Porlamar	NE	VEN	11.01N	74.55W			
61671A	ham	D	235	1			VEN		W			
110372	sea	D	49	1	61 kmSE Juazeiro	BA	BRA	09.54S	40.15W	400	500	
115993	sea	(D)	156	1	110 kmE Ibotirama	BA	BRA	12.23S	42.30W	690	650	
115994	sea	(D)	157	1	113 kmE Ibotirama	BA	BRA	12.24S	42.28W	690	800	
105546B	sea	D	152	1	5 kmE Caetite, on BR030	BA	BRA	14.00S	42.29W			
92838B	sea	(D)	151	1	7 km Serro-Datas	MG	BRA	18.35S	43.24W		800	4.0
104710	sea(cf)	D	42	1	75 kmW Seabra	BA	BRA	12.20S	42.48W	680	800	
110340	sea(cf)	(D)	46	1	11 kmE Barreiras	BA	BRA	12.12S	45.03W	1020	510	
110343	sea(cf)	(D)	47	1	34 kmW Seabra	BA	BRA	12.27S	41.39W	300	1050	
110361	sea(cf)	(D)	155	1	42 kmNE Andarai	BA	BRA	12.33S	41.06W	960	470	
110373	sea(cf)	D	50	1	21 kmNW Morro Chapeu	BA	BRA	11.29S	41.20W	710	1000	
110370B	sea(cf)	D	48	1	7 kmN Palmeiras	BA	BRA	12.40S	41.33W	1000	690	
37037	ham	D	223	5	Santiago		DOM	19.30N	70.42W			
46587	ham	T	225	2	Finca Sto.Tomas		GTM		W			
49080	ham	D	226	2	Golf course, Barranquilla	ATL	COL	11.10N	74.50W			
55821	ham	T	230	2	38 km Maracaibo-San Rafael	ZUL	VEN	10.58N	71.45W	480	30	
56211	ham	(D)	232	2			GLP		W			
61670	ham	D	234	2			VEN		W			
62160	ham	D	240	2	Barranquilla	ATL	COL	11.10N	74.54W	850	100	
70524	ham	(D)	255	2	Florida City	FL	USA	25.27N	80.30W			
73517	ham	(D)	279	2	Craddocks, Nevis		KNA	17.07N	62.35W			
99675	ham	D	143	2	Corozal		PRI	18.39N	66.19W			
109308	ham	D	107	2	16 kmW Barranquilla-Pto Colombia	ATL	COL	10.59N	74.55W	560	10	7.2
109315	ham	D	32	2	Barranquilla, Bocas de Ceniza	ATL	COL	11.04N	74.49W	570	10	7.0
110042	ham	T	34	2	Maracaibo, near Maruma Hotel	ZUL	VEN	10.34N	71.44W	490	66	7.5
110067	ham	D	93	2	Maracaibo, Graduate Ag. School	ZUL	VEN	10.34N	71.44W	490	66	7.5
110068	ham	T	94	2	Maracaibo, Paseo del Lago	ZUL	VEN	10.34N	71.44W	490	66	7.5
110084	ham	D	37	2	Buena Vista	FAL	VEN	11.53N	69.57W	690	70	8.0
110090	ham	D	38	2	22 kmE Churugura-Barquisimeto (H4)	FAL	VEN	10.48N	69.26W	615	708	8.0
110099	ham	D	103	2	Km473, 31 kmS Carora-Agua Viva	LAR	VEN	09.58N	70.06W	731	551	6.5
110179	ham	D	67	2	4 kmSW Carache-La Pastora (Trentino)	TRU	VEN	09.37N	70.16W	569	1545	6.5
110190	ham	D	145	2	San Juan de Lagunillas (H7)	MER	VEN	08.31N	71.21W	542	1100	7.0
40264A	ham	D	53	2		PE	BRA		W			
40264A	ham	D	239	2		PE	BRA		W			
61672Ba	ham	D	236	2			VEN		W			
61672Bb	ham	T	237	2			VEN		W			
55799	sca	(X)	291	2	16 kmSW Crossroads, 21 km Itaberaba	BA	BRA	12.33S	40.31W	580	380	
110359	sca	D	148	2	80 kmNE Agua Viva	LAR	VEN	10.03N	70.03W	740	650	
110341	sea(cf)	(D)	154	2	72 kmE Barreiras	BA	BRA	12.03S	44.24W	1010	750	

40264A	ham	D	185	3		PE	BRA		W			
40264A	ham	D	217	3		PE	BRA		W			
40264A	ham	D	219	3		PE	BRA		W			
ATF2350	sca.affB	(D)	161	3		BA	BRA	10.56S	42.29W	650	500	
92454	sea	(D)	177	3	124 kmE Ibotirama	BA	BRA	12.24S	42.23W	800	900	4.0
92463	sea	(D)	181	3	187 kmE Ibotirama	BA	BRA	12.20S	41.52W	800	1050	
110370C	sea	D	187	3	7 kmN Palmeiras	BA	BRA	12.40S	41.33W	1000	690	
ATF2516	sea	(D)	162	3	Ibotirama to Sitio do Mata	BA	BRA	12.14S	43.13W	690	500	
ATF2517	sea	(D)	188	3	2.2 kmS BR242 - Sitio do Mata	BA	BRA	12.14S	43.13W	690	490	
ATF2518	sea	(D)	189	3	67 kmW Seabra on BR242	BA	BRA	12.27S	42.21W			
ATF2519	sea	(D)	163	3	Cafarnaum	BA	BRA	11.48S	41.31W	680	830	
ATF2520	sea	(D)	190	3	21 kmNW Morro de Chapeu	BA	BRA	11.30S	41.23W			
ATF2522	sea	(D)	192	3	12.8 kmN Wagner-Utinga	BA	BRA	12.08S	41.12W			
ATF2523	sea	(D)	164	3	10.5 kmS BR242 - Andarai	BA	BRA	12.33S	41.07W	960	475	
ATF2530	sea	(D)	193	3	22 kmN BR242 - Wagner	BA	BRA	12.12S	41.04W			
ATF2531	sea	(D)	194	3	11.8 kmN BR242 - Iraquara	BA	BRA	12.12S	41.39W			
ATF2533	sea	(D)	165	3	11.8 kmN BR242 - Iraquara	BA	BRA	12.12S	41.39W		750	
ATF2534	sea	(D)	195	3	3.7 kmN BR242 - Iraquara	BA	BRA	12.15S	41.39W			
ATF2535	sea	(D)	166	3	1.8 kmE Palmeiras jnt.on BR242	BA	BRA	12.30S	41.38W		660	
ATF2536	sea	(D)	196	3	1.8 kmE Palmeiras jnt.on BR242	BA	BRA	12.30S	41.38W			
ATF2537	sea	(D)	167	3	1.8 kmE Palmeiras jnt.on BR242	BA	BRA	12.30S	41.38W		660	
ATF2539	sea	(D)	197	3	1 kmS BR242 - Palmeiras	BA	BRA	12.30S	41.38W			
ATF2540	sea	(D)	199	3	2.4 kmS BR242 - Andarai	BA	BRA	12.30S	41.09W			
110342	sea(cf)	(D)	186	3	124 kmE Ibotirama	BA	BRA	12.24S	42.23W	800	900	4.0

[1] CPI = CSIRO Plant Introduction Number, ATF = CSIRO Australia Tropical Forages Plant Introduction Number.
Numbers underlined = Ha76 series; normal font = Ha160 series; numbers bolded = Ha59 experiments.
Numbers without prefix are CPI accession numbers.

[2] Species: (sp.) = unidentified; cal = *calcicola*; ham = *hamata*; ham.affC = *S.* sp. aff. *S. hamata*-type; hum = *humilis*; sca = *scabra*; sca.affB = *S.*sp. aff. *S. scabra*-type; sea = *seabrana*; sea(cf) = cf *scabra*-type of Maass (1989); sym = *sympodialis*.

[3] Pl = Ploidy: D = diploid; (D) = presumed diploid; T = tetraploid; (T) = presumed tetraploid; (X) = not assessed.

[4] Serial = Unique serial number used to identify accessions throughout screening, data processing and presentation.

[5] Gp = Group number from PATN Analysis classification based on 6 groups from HaAll group with manual re-allocations based on Ha76, Ha160 and Ha59.

[6] S/P = State or Province; Ctry = Country; Lat. = latitude; Long. = longitude; Rain = annual rainfall (mm); Eln = elevation (m).

[7] pH = in water (field kit).

Table 2. List of strains of *Bradyrhizobium* and their provenances that were used to screen accessions of *Stylosanthes*.

Strain	Kruskal-Wallis value[1]			Host[4]	Location	Town	S/P[5]	Country	Latitude	Longitude
# Acc[2] # Str[2]	Ha76[3] 12	Ha160[3] 18	Ha59[3] 11							
CB82	**0.94**	0.75	**0.94**	*S. guianensis*		Fitzroyvale	QLD	Australia	23.22S	153.47E
CB159	0.80	0.05	**0.96**	*D. trilobus*		Maryborough	QLD	Australia	25.32S	152.42E
CB530	0.61	0.76		*A. prostrata*		Samford	QLD	Australia	27.22S	152.53E
CB756	**0.92**	0.01		*M. africanum*	Grassland Research Station	Marandellas		Zimbabwe	18.10S	31.36E
CB1408	**0.98**	0.31		*S. guianensis*				French Guiana		
CB1650	0.71	0.74	**0.98**	*S. guianensis*		Matao	SP	Brazil		
CB2126	0.82	0.01		*S. hamata*	Uni. W. Indies	Kingston		Jamaica		
CB3048		0.65	**0.95**	*S. capitata*		Pariaguan	ANZ	Venezuela	08.54N	64.42W
CB3053	0.86	0.80	**0.98**	*S. hamata*		Shell Beach		Antigua	17.08N	61.48W
CB3050	0.80	0.01		*S. guianensis*		Pto Lopez	MET	Colombia	04.15N	72.30W
CB3289	0.80	0.54	**1.00**	*S. hamata*	Indian Castle	Nevis Is	NEV	Nevis	17.10N	62.40W
CB3290		0.75	**0.97**	*S. hamata*	Old Maracaibo airport	Maracaibo	ZUL	Venezuela	10.44N	71.37W
CB3291	0.78	0.69		*S. hamata*	Uni. Zulia Basketball Stadium	Maracaibo	ZUL	Venezuela	10.44N	71.37W
CB3292		**0.91**	**0.91**	*S. hamata*	Crossroads Routes 1 & 6	Maracaibo	ZUL	Venezuela	10.32N	71.45W
CB3293		0.01		*S. hamata*	East side Maracaibo Bridge	Maracaibo	ZUL	Venezuela	10.33N	72.31W
CB3294	**1.00**	0.72	**0.92**	*S.* (sp.)	13kmW Hwy 4 on F46	Sanford	FLO	United States	28.49N	81.17W
CB3295		**1.00**		*S. capitata*	FONAIAP Research Station		GUA	Venezuela	08.58N	67.28W
CB3296		0.01		*S. capitata*	FONAIAP Research Station		GUA	Venezuela	08.58N	67.28W
CB3480			0.79	*S. seabrana*		Barreiras	BA	Brazil		
CB3481			0.78	*S. seabrana*	1 kmS BR242-Palmeiras	Palmeiras	BA	Brazil	12.35N	41.38W

[1] Kruskal-Wallis Statistic = rank of attribute (strain) contribution to formation of groups; values are proportionate to maximum value for each attribute. **Bolded** values refer to strains contributing most to the accession Group definitions.

[2] # Acc/# Str = number of accessions/strains.

[3] Ha76, Ha160, Ha59 = respectively, Experiments 1981-1982; Experiments 1987-1992; Experiments 1995-1996.

[4] *S* = *Stylosanthes*; *D* = *Dolichos*; *A.* = *Arachis*; *M* = *Macrotyloma*.

[5] S/P = State/Province.

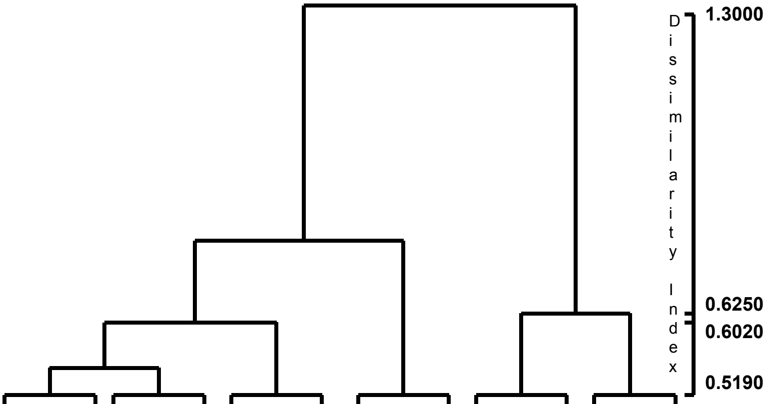
were classified by the hierarchical routine FUSE (UPGMA option). The routines GDEF and DEND were used to display group structure and relationships among groups, and GSTA to determine which attributes (strains) contributed most to the formation of the groups. In addition, MST was used to display dissimilarity between accessions, NNB to determine proximity relationships, BOND to indicate the strength of the relationship between nearest neighbours, and PCA (Principal Components Analysis) to display major groups from each analysis.

Five separate analyses were completed. The index values for the Ha76 experiments, the Ha160 experiments and the Ha59 experiments were analysed separately. Then the HaAll (20% as missing values) and Ha295 (common to all accessions) composite data sets were subjected to separate analysis to provide an overall picture of responses (dissimilarities) of the major groups. Kruskal-Wallis values for the *Bradyrhizobium* strain attributes were calculated from the group structure data (GSTA) to provide a ranking of strains contributing most to the separation of the accessions of *Stylosanthes* into their nitrogen-fixation effectiveness response groups.

Results and Discussion

In all experiments, uninoculated and nitrogen controls remained free of nodules. In 2 experiments, nitrogen controls returned plant dry weights slightly less than the best bradyrhizobial strain treatments.

Pattern analysis of the screening data separated the 295 accessions of *Stylosanthes* into 2 major divisions according to their effectiveness of nitrogen fixation with the range of strains of *Bradyrhizobium*. These divisions comprised groups of accessions that were effectively nodulated by a wide range of strains similar to the promiscuous standard, *S. hamata* cv. Verano (tetraploid), and those that were effectively nodulated by only a few strains of *Bradyrhizobium* typified by *S. hamata* CPI40264A (diploid) (Figures 1, 2, 3 and 4).



Group 1		Group 2	Group 3	Group 4	Group 5	Group 6
33205	72852	37038	65368	37037	61667	46587
33231	72854	65364	65371	49080	61674	55799
36046	73484	70372	73497	56211	68841	55803
50997	73487	73486		61670		55805
50998	73499	73488		62160		55821
51391	73501	73491		70524		65958
55826	73506	73498		73517		65962
62162	73507	73505		40264A		70522
65363	73509	73513		61672Ba		70529
70360	73511	73519				61672Bb
70366	73514	73524				67704B
70370	73515	73525				VERANO
70371	73523	40264A				VERANO
70374	40264A	61623A				VERANO
70523	40264A					VERANO
72850	61671A					

Figure 1. Dendrogram and Group composition for the accessions of *Stylosanthes* in the Ha76 experiments.

Accessions of the diploid *S. hamata* and *S. seabrana* (also diploid) separated into distinct groups within those groups of accessions nodulated effectively by only a few strains of *Bradyrhizobium* (Figures 5 and 6). The percentage variation accounted for in a Principal Components Analysis for each of the analyses shows that most variation is accounted for by the first and second order coordinates, providing strong evidence of significant grouping of accessions with similar responses (Table 3).

The Kruskal-Wallis values for strains of *Bradyrhizobium* contributing most to the grouping of accessions in the 3 series of experiments are recorded in Table 2.

The accessions identified as the ATF2300 series are those described by Maass (1989) as 'aff. scabra-type' (affB by authors to distinguish from affC hamata-type) and they formed 2 main groups with high similarity within the more freely nodulating accessions (Table 1; Group 6 in Figures 3 and 4; Series Ha59 and HaAll in Figure 6). All *S. seabrana* accessions (includes 'cf.-scabra-type' and *S.* sp.aff. *S. scabra*), except CPI92476, CPI115995, ATF2521 and ATF2539B, are identified in Group 1, 2 or 3 of Figures 2, 3 and 4 (also

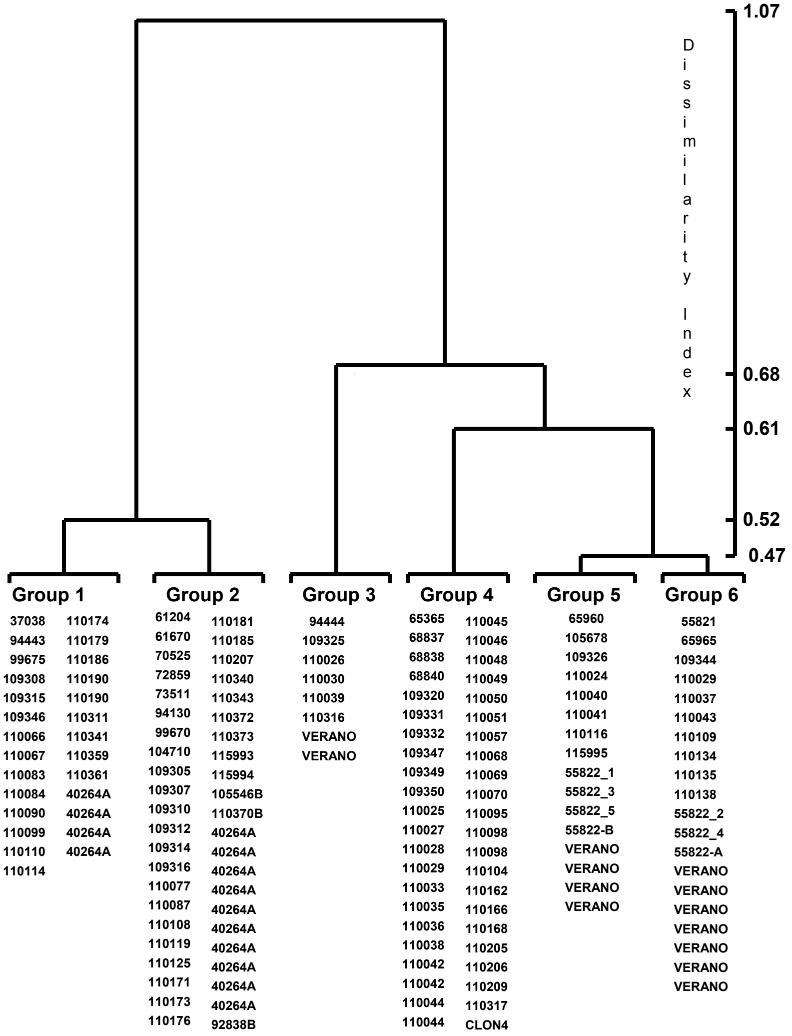


Figure 2. Dendrogram and Group composition for the accessions of *Stylosanthes* in the Ha160 experiments.

Table 1), associated with the diploid specific strain requiring standard CPI40264A. The exceptions occurred in Groups 5 and 6 of Figures 2, 3 and 4 associated with the more promiscuously nodulating standard Verano. Both ATF accessions may not be *S. seabrana*. The morphological data of Date *et al*. (2010) support this suggestion; however, the two CPI accessions are anomalous, as both are distinctly *S. seabrana* in the morphological assessment (Date *et al*. 2010).

With few exceptions in the Ha59 and HaAll series, the accessions in Groups 1 and 2 are diploid and those in Groups 5 and 6 are tetraploid and show the typical rhizobial response patterns of promiscuity for tetraploid accessions and a high level of specificity for the diploid accessions in respect of effectiveness of nodulation by bradyrhizobia. In series Ha76 Groups 1 to 4, the accessions were of the specific strain requiring type but only Groups 1 and 2 showed specificity in the Ha160 series.

Most of the diploid *S. hamata* accessions were collected in regions of heavier clay soils of neutral to alkaline pH and distinct seasonal wet/dry conditions, whereas the tetraploid accessions were from higher rainfall areas and soils of pH <7.

Effective strains of bradyrhizobia from these experiments have been used in glasshouse and field assessment for selection of suitable strains for legume inoculant production. In particular, assessments were completed for the release of the

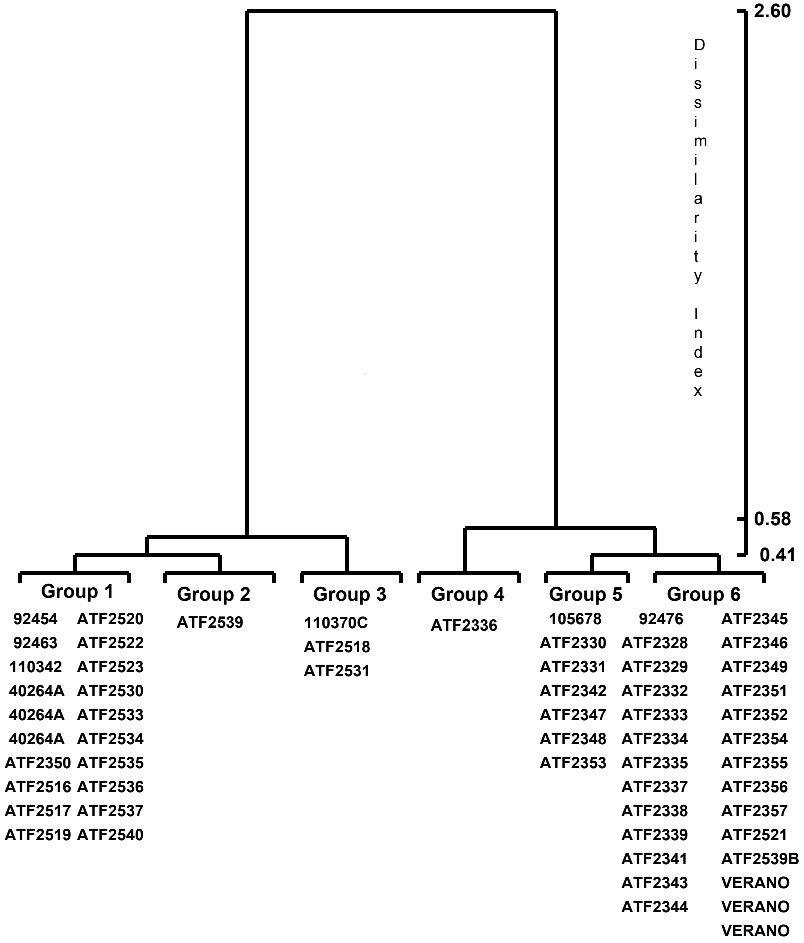
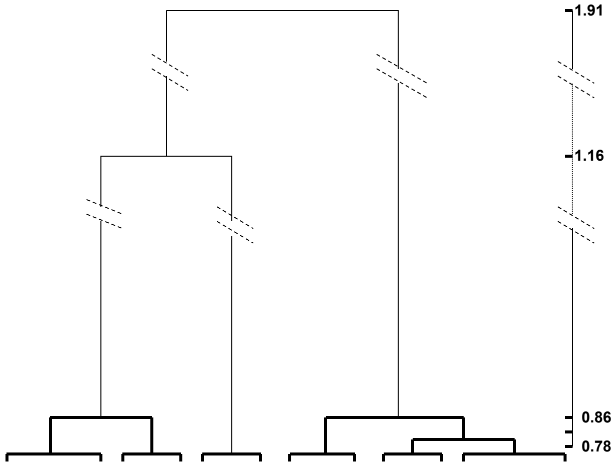


Figure 3. Dendrogram and Group composition for accessions of *Stylosanthes* in the Ha59 experiments.



Group 1

- 33205
- 33231
- 36046
- 37038
- 37038
- 50997
- 50998
- 51391
- 55826
- 61204
- 61670
- 62162
- 65363
- 65364
- 70360
- 70366
- 70370
- 70371
- 70372
- 70374
- 70523
- 70525
- 72850
- 72852
- 72854
- 72859
- 73484
- 73486
- 73487
- 73488
- 73491
- 73497
- 73498
- 73499
- 73501
- 73505
- 73506
- 73507
- 73509
- 73511
- 73511
- 73513
- 73514
- 73515
- 73519
- 73523
- 73524
- 73525
- 94130
- 94443
- 99670
- 104710
- 109305
- 109307
- 109310
- 109312
- 109314
- 109316
- 109346
- 110028
- 110066
- 110077
- 110083
- 110087
- 110108
- 110110
- 110114
- 110119
- 110125
- 110171
- 110173
- 110174
- 110176
- 110181
- 110185
- 110186
- 110190
- 110207
- 110311
- 110340
- 110343
- 110361
- 110372
- 110373
- 115993
- 115994
- 105546B
- 110370B
- 40264A
- 40264A
- 40264A
- 40264A
- 40264A
- 40264A
- 40264A
- 40264A
- 40264A
- 40264A
- 40264A
- 40264A
- 40264A
- 40264A
- 40264A
- 40264A
- 61623A
- 61671A
- 92838B

Group 2

- 37037
- 46587
- 49080
- 55799
- 55821
- 56211
- 61670
- 62160
- 70524
- 73517
- 99675
- 109308
- 109315
- 110042
- 110067
- 110068
- 110084
- 110090
- 110099
- 110179
- 110190
- 110341
- 110359
- 40264A
- 40264A
- 61672Ba
- 61672Bb

Group 3

- 92454
- 92463
- 110342
- 110370C
- 40264A
- 40264A
- 40264A
- ATF2350
- ATF2516
- ATF2517
- ATF2518
- ATF2519
- ATF2520
- ATF2522
- ATF2523
- ATF2530
- ATF2531
- ATF2533
- ATF2534
- ATF2535
- ATF2536
- ATF2537
- ATF2539
- ATF2540

Group 4

- ATF2336

Group 5

- 65365
- 65368
- 65371
- 68837
- 68838
- 68840
- 94444
- 109320
- 109325
- 109331
- 109332
- 109347
- 109349
- 109350
- 110033
- 110035
- 110036
- 110038
- 110042
- 110044
- 110044
- 110045
- 110046
- 110048
- 110049
- 110050
- 110051
- 110057
- 110069
- 110070
- 110098
- 110098
- 110104
- 110162
- 110166
- 110168
- 110205
- 110206
- 110209
- 110317
- CLON4

Group 6

- 55803
- 55805
- 55821
- 61667
- 61674
- 65958
- 65960
- 65962
- 65965
- 68841
- 70522
- 70529
- 92476
- 105678
- 105678
- 109326
- 109344
- 110024
- 110025
- 110026
- 110027
- 110029
- 110029
- 110030
- 110037
- 110039
- 110040
- 110041
- 110043
- 110095
- 110109
- 110116
- 110134
- 110135
- 110138
- 110316
- 115995
- 55822_1
- 55822_2
- 55822_3
- 55822_4
- 55822_5
- 55822-A
- 55822-B
- 67704B
- ATF2328
- ATF2329
- ATF2330
- ATF2331
- ATF2332
- ATF2333
- ATF2334
- ATF2335
- ATF2337
- ATF2338
- ATF2339
- ATF2341
- ATF2342
- ATF2343
- ATF2344
- ATF2345
- ATF2346
- ATF2347
- ATF2348
- TF2349
- ATF2351
- ATF2352
- ATF2353
- ATF2354
- ATF2355
- ATF2356
- ATF2357
- ATF2521
- ATF2539B
- VERANO
- VERANO
- VERANO
- VERANO
- VERANO
- VERANO
- VERANO
- VERANO
- VERANO
- VERANO
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Figure 4. Dendrogram and Group composition for accessions of *Stylosanthes* in the HaAll experiments.

Caatinga stylo (*S. seabrana*) cultivars Primar and Unica (R.A. Date unpublished data).

The accessions of *S. calcicola* were grouped with those of the specific *Bradyrhzobium* strain requiring types. Strains CB2126, CB3050 and CB3053 were the most effective but were less than satisfactory, achieving only 50–55% of the dry weight of the nitrogen controls. *S. humilis* and *S. sympodialis* grouped with the more promiscuously nodulating types. *Bradyrhizobium* strains CB756, CB1650, CB3050 and CB3294 were the most satisfactory, achieving 70–90% dry weights of the nitrogen controls. It is recommended that any further field evaluation of these accessions include a re-assessment of the *Bradyrhizobium* requirements for effective nitrogen fixation.

The experiment series Ha76, Ha160 and Ha59, aimed at selecting strains of bradyrhizobia to nodulate potential new pasture legumes for the seasonally dry tropics in northern Australia, centred on the diploid *S. hamata* and some morphologically different *S. scabra* accessions now recognised as *S. seabrana* (Maass and Mannetje 2002). Initial screening (Ha76 experiments) used the diagnostic strains of Date and Norris (1979). Changes were made after these strains failed to separate accessions and more importantly when no strain of potentially high level effectiveness was identified. The screening was widened in the Ha160 series and, when none of the *S. seabrana* material was effectively nodulated in this series of experiments, it was broadened again (Ha59 series). The Ha160 series used strains of bradyrhizobia obtained from a specifically targeted collection of new material from Venezuela (strains CB3289, CB3290, CB3291, CB3292, CB3293, CB3294, CB3295 and CB3296) to correspond with the diploid *S. hamata* accessions. The Ha59 experiments included strains CB3480 and CB3481 to correspond with the new germplasm of *S. seabrana* (ATF2500 series). Strain

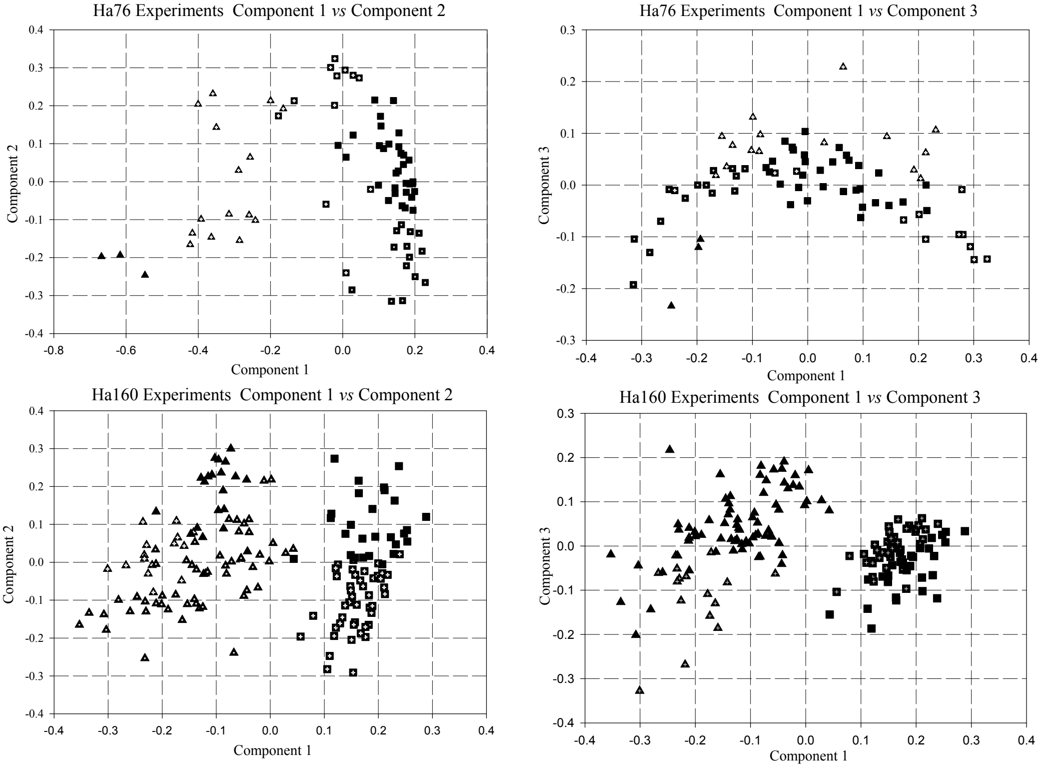

Figure 5. Scatter diagrams of components 1, 2 and 3 for Experiment series Ha76 and Ha160. Triangles = promiscuous; squares = specific.

CB3481 has subsequently become the commercial inoculant for *S. seabrana* in Australia (Bullard *et al.* 2005). Although strain CB3481 is effective and persistent as an inoculum for *S. seabrana* in the field (R.A. Date unpublished data), strains CB3480, CB3292 and CB3294 were more effective in these screening experiments and warrant field assessment for their competitive and persistence abilities. Similarly, strains CB2126 and CB3050, suitable for diploid *S. hamata,* should be assessed if accessions of this species are included in future regional evaluation trials. In addition, *Bradyrhizobium* strains CB3495 and CB3564, which were not available for the Ha76, Ha160 and Ha59 experiments, warrant inclusion in future assessment of both diploid *S. hamata* and *S. seabrana*. Strain CB3495 is effective in the field (Eagles and Date 1999) and strain CB3564 was isolated from *S. seabrana* and has proven effective on *S. macrocephala* (Date and Eagles 2010).

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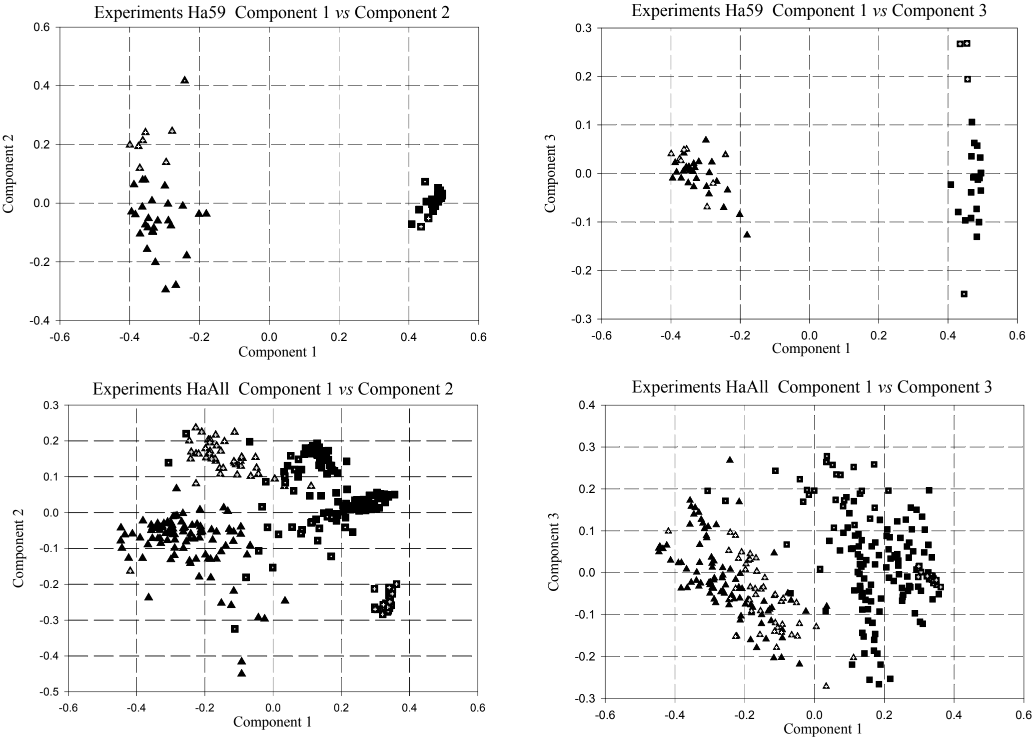

Figure 6. Scatter diagrams of components 1, 2 and 3 for Experiment series Ha59 and HaAll. Triangles = promiscuous; squares = specific.

Table 3. Percentage of variation accounted for by Principal Components Analysis of effectiveness response data for the 3 series of experiments assessing nitrogen-fixation effectiveness of accessions of *Stylosanthes* against a range of strains of *Bradyrhizobium*.

Series	Component 1	Component 2	Component 3	Total variation
Ha76	51	27	6	84
Ha160	44	26	12	82
Ha59	83	8	4	95
HaAll	58	20	12	90

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