# *Bradyrhizobium* strain effectiveness for *Stylosanthes macrocephala*

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**Abstract**

The effectiveness of 31 strains of *Bradyrhizobium* in producing nodules on and fixing nitrogen with 31 accessions of *Stylosanthes macrocephala* was assessed in 3 experiments using nitrogen-free sand-culture conditions in a glasshouse. Most strains of *Bradyrhizobium* isolated from *S. macrocephala* formed effective nitrogen-fixing associations with the range of *S. macrocephala* accessions. Variability of the responses was greater with strains of bradyrhizobia isolated from *S. seabrana,* which has a similar geographic distribution, and with strains isolated from *S. capitata,* which has the same geographic distribution and is botanically closely related.

## Introduction

In Australia during the 1980s and 1990s there was considerable interest in expanding the range of *Stylosanthes*-based cultivars for pasture improvement and *Stylosanthes macrocephala* was thought to have potential for infertile soils in the seasonally dry tropical/subtropical regions. *S. macrocephala* occurs naturally in Brazil (Schultze-Kraft *et al*. 1984; Williams *et al*. 1984) and is found in a range of climates and habitats. The majority of CSIRO's germplasm collection of *S. macrocephala* originated in acid infertile soils, but some were found on near neutral clay-loam soils. On the basis of provenance data for 55 accessions of *S. macrocephala,* Schultze-Kraft *et al*. (1984) recognised 7 geographical regions within Brazil; however, there are no published systematic morphological descriptions of these germplasm collections. Some initial grouping based on morphological and agronomic characteristics was attempted in the late 1980s for the germplasm held by CSIRO in Australia (R.J. Williams, personal communication) but, in the field, many of the accessions soon showed symptoms of nitrogen deficiency. Subsequent examination failed to locate any nodules (author observation). Schultze-Kraft *et al*. (1984) assessed agronomic potential of *S. macrocephala* in field experiments in Sete Lagoas, Brazil, and in Quilichao, Colombia, but did not refer to either inoculation or nitrogen-fixation aspects. Sete Lagoas is located within the natural distribution area of *S. macrocephala* (Williams *et al*. 1984). The cultivar 'Pioniero' was released in Brazil in the early 1980s (Sousa Costa and Ferreira 1984). In regional small plot evaluation trials in Brazil, *S. macrocephala* produced high dry matter and seed yields (Fernandes *et al*. 2004) and, as a component of grass-stylo pastures in drier environments, the cultivar Campo Grande (a mixture of *S. macrocephala* and *S. capitata*) has supported encouraging levels of animal production (Andrade *et al*. 2004). In field evaluation of a limited number of accessions in southern Queensland, there was good establishment on a sandy acid (pH 5.5) soil (Hall and Glatzle 2004).

An important aspect of a forage legume is its capacity to provide high-quality forage via its ability to provide nitrogen through effective nodulation. This paper reports results of glasshouse experiments aimed at determining *Bradyrhizobium* specificity for effective nitrogen fixation for part of CSIRO's *S. macrocephala* collection.

## Materials and methods

### *Germplasm*

Three separate screening experiments involving 31 accessions of *S. macrocephala* (Table 1),

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representing the geographic range of accessions in the CSIRO germplasm collection, were tested against a range of strains of *Bradyrhizobium* (Table 2) for their ability to form nodules and fix nitrogen. The accessions of *S. macrocephala* used in these experiments originated from Regions I, III, V and VI of Schultze-Kraft *et al*. (1984). Selection for assessment from the CSIRO collection was based on seed availability and representative morphological ecotypes with agronomic potential as defined by R.J. Williams (personal communication, see Table 1).

Experiment 1 (1991) used 3 accessions and 17 strains of bradyrhizobia (refer Tables 1 and 2). The accessions used represented 2 of the areas (Distrito Federal and Minas Gerais) defined by Schultze-Kraft *et al*. (1984). At the time of the screening, no strains isolated from *S. macrocephala* were held in the CSIRO *Rhizobium* Germplasm Collection. In a previous study of strains of bradyrhizobia and their nitrogen-fixation effectiveness, specificities with other species of *Stylosanthes* (Date and Norris 1979) identified a small number of strains as effective at fixing nitrogen with a wide range of accessions. They referred to these as diagnostic strains. One of them, CB1650, has its origin in the same geographic region of Brazil as the *S. macrocephala* in these experiments. The remainder are 'best-guess' selections either originating from the same geographic areas as *S. macrocephala* and/or isolated from the closely related *S. capitata* with which it shares a similar geographic distribution. By 1997, additional strains of bradyrhizobia isolated from *S. macrocepahala*, *S. seabrana* and *S. capitata* and seed supplies of additional accessions of *S. macrocephala* became available.

**Table 1.** Geographic origin and regional groups of accessions of *Stylosanthes macrocephala* used in Experiments 1, 2 and 3.

| Accession CPI | State[1] (Brazil) | SK[2] Region | RJW[3] Group | Exp 1 | Exp 2 | Exp 3 |
|---|---|---|---|---|---|---|
| 54835 | BA | III or VI | 7 | | y | y |
| 75179 | DF | I | 2 | | y | y |
| 92451 | BA | III | 9 | | y | y |
| 92626 | MG | VI | 13 | | | y |
| 92853 | MG | VI | 20 | | | y |
| 92860 | MG | VI | 11 | | y | y |
| 92920 | DF | I | 3 | | y | y |
| 93037 | SP | ( ) | 10 | | y | y |
| 93048 | MG | VI | | | | y |
| 93064 | MG | VI | 19 | | | y |
| 93101 | MG | VI | 2 | | y | y |
| 94404 | DF | I | 3 | y | y | y |
| 105458 | MG | VI | 18 | | y | y |
| 105460 | MG | VI | 26 | | | y |
| 105462 | DF | I | 17 | | y | y |
| 105463 | DF | I | 3 | | y | y |
| 105465 | (BA-MG) | (V or VI) | 5 | | y | y |
| 105471A | (BA-MG) | (V or VI) | 25 | | | y |
| 105473 | (BA-MG) | (V or VI) | 15 | | | y |
| 105481 | DF | I | 15 | y | y | y |
| 105494 | MG | V or VI | 19 | y | | y |
| 105496C | BA | VI | 21 | | | y |
| 105497 | BA | VI | 10 | | y | y |
| 105499 | (MG) | (VI) | 13 | | | y |
| 105503 | (MG) | (VI) | 2 | | y | y |
| 105504P | (MG) | (VI) | 11 | | | y |
| 106880 | GOI | I or III | 4 | | y | y |
| 106884 | MG | VI | 10 | | y | y |
| 106885 | MG | VI | 4 | | | y |
| 106894 | MG | VI | 8 | | | y |
| 106896 | MG | V or VI | 1 | | | y |
| Verano | | | | | y | y |

[1] State of Brazil: BA = Bahia; DF = District Federal; MG = Minas Gerais; SP = Sao Paulo; GOI = Goias; ( ) = best estimate based on limited provenance data.

[2] SK Region = from Schultze-Kraft *et al*. (1984).

[3] RJW = Morphological/Agronomic groups of R.J. Williams.

In Experiment 2, the ability of 17 strains of bradyrhizobia, which included 2 of the key diagnostic strains (CB82 and CB1650) from Date and Norris (1979) and the best performing strain (CB3055) from Experiment 1, to produce nodules on and fix nitrogen with 17 accessions of *S. macrocephala*, was evaluated.

Experiment 3 used only 7 strains of bradyrhizobia considered to be sufficiently diagnostic [levels of effectiveness from ineffective (i) to highly effective (HE), see Table 3] to assess any variability in N-fixation response of 31 accessions of *S. macrocephala*. The commercial cultivar Verano (*S. hamata*) was included for comparison.

*Plant growth conditions and measurements*

Pregerminated 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 5-7 days after sowing by adding a 1 ml broth suspension of the appropriate strain of *Bradyrhizobium* (approximately $10^9$ cells). 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°C. Plants were harvested after 8-10 weeks. Roots were

**Table 2**. Host species and geographic origins of *Bradyrhizobium* used in Experiments 1, 2 and 3.

| Strain | Host of origin[1] | Exp 1 1991 | Exp 2 1997 | Exp 3 1999 | Country[2] | State[3] |
|---|---|---|---|---|---|---|
| CB82 | gui | | ✓ | | AUS | QLD |
| CB1650 | gui | ✓ | ✓ | ✓ | BRA | SP |
| CB2898 | cap | ✓ | | | AUS | QLD |
| CB3048 | cap | ✓ | ✓ | | VEN | ANZ |
| CB3049 | cap | ✓ | | | VEN | ANZ |
| CB3050 | gui | ✓ | | | COL | VIC |
| CB3052 | gui | ✓ | | | COL | VIC |
| CB3055 | sp. | ✓ | ✓ | ✓ | BRA | BA |
| CB3154 | cap | ✓ | | | BRA | GOI |
| CB3214 | cap | ✓ | | | BRA | GOI |
| CB3241 | cap | ✓ | | | BRA | AM |
| CB3242 | cap | ✓ | | | BRA | AM |
| CB3294 | sp. | ✓ | | | USA | FL |
| CB3304 | cap | ✓ | | | COL | CAU |
| CB3305 | cap | ✓ | | | VEN | MON |
| CB3306 | cap | ✓ | | | VEN | ANZ |
| CB3307 | cap | ✓ | | | VEN | ANZ |
| CB3308 | cap | ✓ | | | VEN | ANZ |
| CB3481 | sea | | √ | | BRA | BA |
| CB3482 | mac | | ✓ | | BRA | BA |
| CB3557 | mac | | ✓ | | BRA | MG |
| CB3558 | mac | | ✓ | | BRA | MG |
| CB3559 | mac | | ✓ | | BRA | MG |
| CB3560 | sp. | | ✓ | ✓ | BRA | MG |
| CB3561 | vis | | ✓ | ✓ | BRA | MG |
| CB3562 | mac | | ✓ | | BRA | BA |
| CB3563 | sea | | ✓ | ✓ | BRA | BA |
| CB3564 | sea | | ✓ | ✓ | BRA | BA |
| CB3565 | mac | | ✓ | | BRA | BA |
| CB3566 | mac | | ✓ | ✓ | BRA | BA |
| CB3567 | cap | | ✓ | | BRA | GOI |

[1]cap = *S. capitata*; gui = *S. guianensis*; sp. = *S.* species; mac = *S. macrocephala*; sea = *S. seabrana*; vis = *S. viscosa*.

[2] AUS = Australia; BRA = Brazil; COL = Colombia; USA = United States of America; VEN = Venezuela.

[3]AM = Amazonas; ANZ = Anzoategui; BA = Bahia; CAU = Cauca; FL = Florida; GOI = Goias; QLD = Queensland; VIC = Vichada; MG = Minas Gerais, MON = Monagas.

washed free of sand and nodulation recorded as: '-' (none), '+' (few) or '++' (many). The dry weights of whole plants (4/sand-jar) were used as an index of the effectiveness of nitrogen fixation.

*Data analysis*

Plant dry weights were standardised by expressing values as a percentage of the relevant nitrogen control values, since the purpose of the screening was to assess: relative responses in nitrogen-fixation effectiveness to a group of strains of bradyrhizobia; and any variability in this response between accessions of *S. macrocephala*. The index values (dry weights, Experiment 3) for the 31 x 9 data matrix were subjected to pattern analysis using PATN (Belbin 1995) to allocate like response patterns to a limited number of groups. 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 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 and BOND to indicate the strength of the relationship between nearest neighbours.

## Results

*Experiment 1*

Only 4 strains (CB2898, CB3048, CB3049 and CB3055) were effective in fixing nitrogen with the *S. macrocephala* accessions, but level of effectiveness varied with the accession (Table 3). In all other combinations, nodules were formed but the associations were ineffective in nitrogen fixation.

*Experiment 2*

All 17 strains of bradyrhizobia formed nodules with *S. hamata* cv. Verano but surprisingly strain CB3557, which was isolated from *S. macrocephala*, did not produce nodules on any accession of *S. macrocephala* (Table 4). Strains CB3558 and CB3565, also from *S. macrocephala*, formed nodules with only 2 and 8, respectively, of the 17 accessions; however, CB3565 was effective (HE) with 4 of the accessions (Table 4). Strain CB3563 formed nodules on 16 of the accessions but was ineffective in nitrogen fixation with 15 of them. Strains CB3055, CB3561, CB3562, CB3564, CB3566 and CB3567 formed effective associations with 12 - 14 of the accessions, but only CB3562 and CB3566 were isolated from *S. macrocephala*.

*Experiment 3*

As in Experiment 2, all strains of bradyrhizobia formed nodules with *S. hamata* cv. Verano. Strain CB3563 failed to form nodules on 16 of the 31 accessions of *S. macrocephala.* Strains CB3055, CB3561, CB3564 and CB3566 formed effective symbioses with all accessions of *S. macrocephala*, while strains CB1650, CB3560 and CB3563 were ineffective (Table 5).

*Analyses*

Three major similarity groups resulted from the grouping pattern analysis (Figure 1). In Groups I and II, responses were reasonably uniform within

**Table 3.** Nitrogen-fixation effectiveness[1] responses of 3 accessions of *S. macrocephala* and 4 strains of *Bradyrhizobium* in Experiment 1.

| Accession/Strain | CB2898 | CB3048 | CB3049 | CB3055 |
|---|---|---|---|---|
| 94404 | HE | e | e | HE |
| 105481 | HE | HE | HE | HE |
| 105494 | e | e | E | I |

[1]HE = >100% of N control, E = 81-100%, e = 51-80%, I = 31-50%, i = 0-30% (see text).

Groups. In Group III, accession CPI 54835 was effective (E) with only 2 of the 7 strains of bradyrhizobia, CPI 106885 was highly effective (HE) with the same 4 strains as in Groups I and II but responded in different proportions, resulting in its placement in a separate group with CPI 54835.

## Discussion

These screening trials have indicated that, for effective nitrogen fixation, *S. macrocephala* has a moderate level of specificity for strains of *Bradyrhizobium*. Although *S. macrocephala* is closely related botanically and geographically to *S. capitata*, bradyrhizobia isolated from *S. capitata* did not necessarily fix nitrogen with *S. macrocephala,* nor did bradyrhizobia isolated from *S. macrocephala* growing in the same or different regions. For example, bradyrhizobial strain CB3482, from western Bahia (near Barreiras), formed effective (HE and E) associations with only 9 of the 17 accessions in Experiment 2, whereas strain CB3566 from the same location formed HE and E associations with 14 of the 17 accessions in Experiment 2, and 29 of the 31 accessions in Experiment 3. One accession CPI 54835 was ineffectively (I) nodulated in both experiments.

Effectiveness response groups were not associated with region of origin, either by state or

**Table 4.** Nitrogen-fixation effectiveness[1] responses of 17 accessions of *S. macrocephala* and 17 strains of *Bradyrhizobium* in Experiment 2.

| Strain | Number of accessions | | | |
|---|---|---|---|---|
| | HE and E | e and I | i | not nodulated |
| CB82 | 0 | 0 | 11 | 6 |
| CB1650 | 0 | 0 | 12 | 5 |
| CB3048 | 1 | 2 | 7 | 7 |
| CB3055 | 12 | 4 | 1 | 0 |
| CB3481 | 2 | 0 | 7 | 8 |
| CB3482 | 9 | 5 | 3 | 0 |
| CB3557 | 0 | 0 | 0 | 17 |
| CB3558 | 0 | 0 | 2 | 15 |
| CB3559 | 6 | 7 | 3 | 1 |
| CB3560 | 4 | 4 | 7 | 2 |
| CB3561 | 14 | 1 | 2 | 0 |
| CB3562 | 12 | 2 | 3 | 0 |
| CB3563 | 0 | 1 | 15 | 1 |
| CB3564 | 14 | 2 | 1 | 0 |
| CB3565 | 4 | 1 | 3 | 9 |
| CB3566 | 14 | 2 | 1 | 0 |
| CB3567 | 13 | 3 | 1 | 0 |

[1]HE = >100% of N control, E = 81-100%, e = 51-80%, I = 31-50%, i = 0-30% (see text).

**Table 5.** Nitrogen-fixation effectiveness[1] responses of 31 accessions of *S. macrocephala* and 7 strains of *Bradyrhizobium* in Experiment 3.

| Strain | Number of accessions | | | |
|---|---|---|---|---|
| | HE and E | e and I | i | not nodulated |
| CB1650 | 0 | 0 | 31 | 0 |
| CB3055 | 26 | 5 | 0 | 0 |
| CB3560 | 0 | 3 | 27 | 1 |
| CB3561 | 25 | 4 | 2 | 0 |
| CB3563 | 0 | 2 | 13 | 16 |
| CB3564 | 29 | 1 | 1 | 0 |
| CB3566 | 29 | 2 | 0 | 0 |

[1]HE = >100% of N control, E = 81-100%, e = 51-80%, I = 31-50%, i = 0-30% (see text).

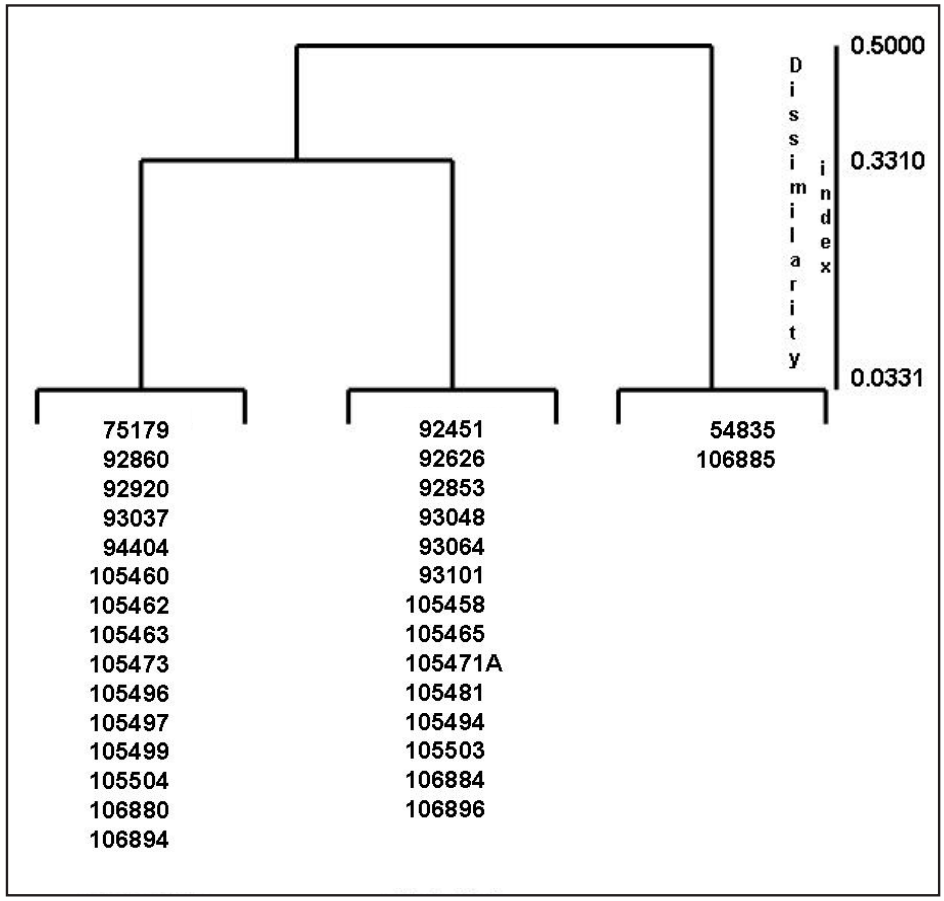


**Figure 1**. Dendogram of groups from PATN grouping of 31 accessions of *Stylosanthes macrocephala* from Experiment 3.

those regions defined by Schultze-Kraft *et al*. (1984). The geographic origins of most of the 31 accessions of *S. macrocephala* coincide with Regions I and VI of Schultze-Kraft *et al*. (1984) and several may be duplicate accessions. We are unable to confirm this from the germplasm database records.

Using RAPD markers, Barros *et al*. (2005) demonstrated limited grouping of 87 accessions of *S. macrocephala* to hydrographic regions and that accessions collected in the states of Bahia and Minas Gerais had the highest variability. Given that most of the 31 accessions assessed for nitrogen-fixation responses to strains of bradyrhizobia have provenances in Bahia and Minas Gerais, it is not surprising that a similar level of diversity was observed. Regrettably, none of the 31 accessions coreresponded with those of Barros *et al*. (2005). Similarly, there was no relationship with the morphological-agronomic groups of R.J. Williams (personal communication). Definition of these groups is not available but there was no consistent grouping or arrangement of the allocated group numbers.

Schultze-Kraft *et al*. (1984) reported that plants at both Sete Lagoas and Quilichao "grew normally and plants did not show any symptoms of mineral deficiency or Al toxicity". This is in contrast with observations on the granitic sandy soils of the CSIRO Narayen Field Station, Queensland, where obvious symptoms of nitrogen deficiency were apparent after the first season. The data in this paper suggest that future evaluation trials of *S. macrocephala* should be preceded by trials designed to test the field competence of several of the HE and E strains of

bradyrhizobia both for their ability to form effective nitrogen-fixing nodules in the season of sowing and for persistence in the soil over several growing seasons.

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