VERIFICATION OF AND GENETIC VARIATION BETWEEN *STYLOSANTHES* SP. AFF. S. SCABRA ACCESSIONS

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ABSTRACT
Thirty-three *Stylosanthes* accessions, which are all considered to be *S*. sp. aff. *S. scabra*, were analysed using STS and RAPD as genetic markers. These accessions were readily clustered into two major groups based on dissimilarity values, with 28 accessions in one group (A) and the other five in the other (B). STS analysis indicated that accessions in group A are diploid *S*. sp. aff. *S. scabra* but those in B are tetraploids, likely to be *S. scabra* or its closely related taxa. Cytological examination of root-tip cells confirmed that the five accessions in group B are all tetraploids. Genetic dissimilarity among the 28 accessions in group A was 0.094, that among the five accessions in group B was 0.137, and that between the two groups was 0.549. The taxonomic status of *S*. sp. aff. *S.* was discussed.

KEYWORDS
Stylosanthes, Stylosanthes sp. aff. *S. scabra*, molecular markers

INTRODUCTION
Stylosanthes sp. aff. *S. scabra* is an undescribed taxon which resembles *S. scabra* in many characters including fruit shape (Edye and Hall 1993) which is one of the few key morphological characteristics used for classification at the species level in *Stylosanthes* (Mannetje 1984). Accessions from this group of plants show potential as forage for tropical and subtropical regions of northern Australia with clayey soils (Edye and Hall 1993; Date et al. 1996). Cytological and STS (sequence-tagged-sites) analyses of the original 13 accessions held at the Australian Tropical Forages Genetic Resource Centre (ATFGRC) showed them to be diploids and likely to be a progenitor of *S. scabra* (Liu and Musial 1996). To extend the number of accessions of *S*. sp. aff. *S. scabra*, a collecting trip was undertaken by staff from CSIRO DTCP in 1994. As a result, the number of the accessions considered to be *S*. sp. aff. *S. scabra* held at ATFGRC was increased to 34. As part of an evaluation program, 33 of these accessions were analysed using molecular marker techniques, aiming to verify the identity of, and assess genetic variation between, the *S*. sp. aff. *S. scabra* accessions.

MATERIALS AND METHODS
Seeds of all the 34 accessions (all considered to be *S*. sp. aff. *S. scabra*) held at ATFGRC (Table 1) were germinated. Three to five seedlings (4- to 7-day-old) were used for DNA extractions following the methods described by Liu (1996). DNAs suitable for PCR analysis were obtained from all but one (ATF 2536) of these accessions.

RAPD analysis was performed using 23 oligonucleotide primers obtained from Operon Technologies, Inc. They include AA-10, AA-16, AA-19, AB-3, AI-3, AI-5, AI-12, AL-3, AL-12, AL-13, AL-18, AN-14, B-5, B-7, C-11, G-6, G-16, G-14, H-17, I-16, L-17, T-3 and T-8. Procedures of PCR analysis and data processing were those described by Liu (1996). STS (using primer pair SHSF3/R3) and cytological analyses were performed following the method described by Liu and Musial (1996).

RESULTS
More than 300 RAPD fragments were generated among the 33 accessions by the 23 oligonucleotide primers. However, only 72 fragments could be reliably scored. Of them, 62 showed polymorphism between at least one pairwise comparison among the 33 accessions, and the remaining 10 were monomorphic.

Cluster analysis based on the 72 RAPD fragments separated the 33 accessions into two major groups (Fig. 1, Table 1). Twenty-eight of them fell into one group (A) and the other five fell into the other (B). The average dissimilarity (1-F) value among the 28 accessions in group A was 0.094 and that among the five accessions in subgroup B was 0.137. The average 1-F value between accessions in the two groups was 0.549, which was even higher than those obtained between *Stylosanthes* species (Kazan et al. 1993). The five accessions in group B were analysed together with four accessions in group A using STS with SHSF3/R3 as primers. A typical *S*. sp. aff. *S. scabra* banding pattern (Liu and Musial 1996) was produced by each of the four accessions from group A. However, the five accessions from group B all produced a two-banded STS pattern similar to that produced by *S. scabra* (Fig. 2). Cytological examination of root-tip cells confirmed that all the five accessions in group B are tetraploids, i.e., containing 40 chromosomes (not shown).

DISCUSSION
Of the 33 accessions analysed, 28 were shown to be diploid *S.* sp. aff. *S. scabra*. The other five are tetraploids, presumably belonging to *S. scabra* or its closely related taxa. The level of polymorphism among the 28 *S*. sp. aff. *S. scabra* accessions obtained in this study (0.094) is comparable to those obtained from other *Stylosanthes* species (Kazan et al. 1993), despite the fact that all the *S.* sp. aff. *S. scabra* accessions were collected in a small area of east central Brazil (Date et al., 1996).

Identification of five tetraploid genotypes from the 33 accessions demonstrated that it is difficult to consistently identify *S*. sp. aff. *S. scabra* from *S. scabra* and its closely related taxa based on morphological characters. So, according to traditional views (e.g. Martin 1996), *S*. sp. aff. *S. scabra* should not be classified as a distinct species. However, *S. scabra* is an allotetraploid but *S*. sp. aff. *S. scabra* is a diploid with the latter being one of the two putative progenitors of the former (Liu and Musial 1996). Due to their different ploidy levels, these two groups of plants should be reproductively isolated. Thus, biologically, *S*. sp. aff. *S. scabra* and *S. scabra* are two different species.

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Table 1
Stylosanthes genotypes used in this study

Series No	Genotype	Series No	Genotype	Series No	Genotype	Series No	Genotype
V1	ATF 2516	V10	ATF 2533	V18	CPI 92463	V27	CPI 110361
V2	ATF 2517	V11	ATF 2534	V19	CPI 92476	V28	CPI 110370C
V3	ATF 2518	V12	ATF 2535	V20	CPI 92838B	V29	CPI 110372
V4	ATF 2519	V13	ATF 2537	V21	CPI 105546B	V30	CPI 110373
V5	ATF 2520	V14	ATF 2539	V22	CPI 105678	V31	CPI 115993
V6	ATF 2521	V15	ATF 2539B	V23	CPI 110340	V32	CPI 115994
V7	ATF 2522	V16	ATF 2540	V24	CPI 110341	V33	CPI 115995*
V8	ATF 2523	V17	CPI 92454	V25	CPI 110342	V34	ATF 2536
V9	ATF 2530			V26	CPI 110343		

*: Possibly a mixture of morphological types. Two selections of this accession have been identified to be *S.* sp. aff. *S. scabra*.

Figure 1
Clustering of 33 *Stylosanthes* genotypes based on RAPD data