SEXUALITY AND HYBRIDIZATION IN SIGNAL GRASS, *BRACHIARIA DECUMBENS*

J. B. HACKER

CSIRO Division of Tropical Crops and Pastures, 306 Carmody Road, St Lucia, Queensland 4067.

ABSTRACT

Brachiaria decumbens, *a widely-sown tropical pasture grass, is an obligate apomict and genetic improvement by breeding is not possible in the absence of a sexual form. Forty accessions of the genus* Brachiaria, *including 14 species, were grown as spaced plants in a field experiment. Plots were assessed for morphological variation which might be associated with sexuality.*

A single accession of B. decumbens *exhibited morphological variation and genetic variation was further demonstrated using starch gel electrophoresis. The accession proved to be a diploid. When pollinated with pollen from a naturalized population of tetraploid* B. decumbens *a triploid hybrid was produced. Chromosome pairing at meiosis showed the genome from the diploid was homologous with 1 genome of the male parent. Chromosomes of the other genome failed to pair, resulting in total male and female sterility.*

Successful utilization of this novel source of sexuality in a breeding program will require chromosome doubling of the hybrid or of the diploid sexual parent prior to hybridization.

RESUMEN

Brachiaria decumbens *es una gramínea tropical ampliamente cultivada, es una especie apomítica obligada y no es posible mejorarla genéticamente por cruzamiento en la ausencia de una forma sexual. Cuarenta accesiones del género* Brachiaria, *incluyendo 14 especies, fueron cultivadas como plantas individuales en un experimento de campo. Las parcelas fueron evaluadas por su variación morfológica asociable con sexualidad.*

Una accesión individual de B. decumbens *mostró variación morfológica y genética, lo que fue demostrado usando electroforesis en gel de almidón. La accesión probó ser diploide. Cuando se polinizó con polen de una población natural de* B. decumbens *tetraploide se produjo un hibrido triploide. Los cromosomas pareados en la meiosis mostraron que el genoma del diploide fue homólogo con un genoma del padre. Los cromosomas del otro genoma no fueron pareados, resultando en macho y hembra totalmente estériles.*

La utilización exitosa de esta fuente de sexualidad en programa de mejoramiento genético requerirá de la duplicación de cromosomas del hibrido resultante o del padre sexual diploide previamente a la hibridización.

INTRODUCTION

Brachiaria decumbens is a grass of East African origin which has achieved significance as a pasture species in many countries in the tropics and subtropics (Bogdan 1977), including northern Australia (Loch 1977). The species is an aposporous apomict, with a chromosome number of 2n = 4x = 36 (Pritchard 1967). Although no publications have been sighted describing variability within *B. decumbens*, various related taxa appear in the literature including *B. eminii*, *B. ruziziensis* and *B. brizantha* which have variously been regarded as synonoms or segregates of *B. decumbens* or which intergrade into that species (see Loch 1977; Clayton and Renvioze 1982). *B. ruziziensis* was considered by Bogdan (1959) to be apomictic, as it bred true to type when heads were enclosed in bags. However more

recent reports indicate that most collections are sexual diploids (Ferguson and Crowder 1974). Chromosome number of diploid sexual *B. ruziziensis* has successfully been doubled using colchicine (Gobbe *et al.* 1981; Swenne *et al.* 1981) and the autotetraploid has been hybridized with *B. decumbens* and *B. brizantha* (Gobbe *et al.* 1983).

Genetic improvement of *B. decumbens* requires the identification of sexual genotypes and their hybridization with agronomically desirable apomicts. Further, sexual genotypes may potentially be utilized as bridges to recombine useful characters present in different apomicts. This would be achieved by transferring a potentially useful gene from an apomict to a sexual background by hybridization, then transferring to the target apomict, again by hybridization. The work described in this paper covers a search for sexuality in *Brachiaria*, the identification of a sexual genotype of *B. decumbens* and its hybridization with apomictic *B. decumbens*.

EXPERIMENTAL

The search for sexuality

The entire *Brachiaria* collection held by the Australian Tropical Forages Genetic Resources Centre, Brisbane, was planted at Lawes, S.E. Queensland. The total number of accessions numbered 40 and included taxa listed as *B. dictyoneura*, *B. dura*, *B. decumbens*, *B. marlotii*, *B. brizantha*, *B. deflexa*, *B. plantagenia*, *B. erecta*, *B. ruziziensis*, *B. nigropedata*, *B. humidicola*, *B. serrifolia* and *B. xantholeuca*. Each accession was represented by up to 24 plants grown from seed and spaced 1 m apart within and between rows. Plants were examined periodically for evidence of intra-accession variation. However, in several accessions the small number of plants precluded recognition of variation.

Only in CQ 3370 was there evidence of variation. Individual plants differed in growth habit and flowering time. As the plants were strongly stoloniferous they had grown into each other before flowering and it was only practicable to harvest a bulk seed sample from the 11 available plants. This accession is of uncertain origin. Using existing keys it is tentatively assigned to *B. decumbens*, although it differs from typical representatives of that species in its strongly decumbent-stoloniferous habit, its low stature and its short racemes. It also differs markedly from *B. ruziziensis* which is described *inter alia* as having the raceme rachis 3-5 mm wide, spikelets 6 mm long and racemes 10 cm long (Germain and Evrard 1953). CQ 3370 has smaller spikelets, shorter racemes and a narrower raceme rachis (Table 1) and also lacks the pronounced space on the rachilla between upper and lower glume characteristic of *B. ruziziensis*. Voucher herbarium specimens have been lodged in the Queensland Herbarium.

TABLE 1

Characteristics of CQ 3370, naturalized Brachiaria decumbens *and their triploid hybrid. (Inflorescence characters are based on means from 15 panicles sampled from field-grown plants).*

	CQ 3370	Hybrid	Naturalized *B. decumbens*
Racemes per panicle	4.6 (3–7)	4.3 (3–6)	4.6 (3–8)
Maximum raceme length (mm)	42 (30–58)	96 (80–110)	98 (80–125)
Spikelet rows on a raceme	2.0 (—)	1.89 (1.8–2.0)	1.97 (1.8–2.0)
Spikelet length (mm)	4.1	4.8	4.8
Lower glume length/spikelet length	0.43	0.51	0.49
Width of raceme axis (mm)	1.3	1.6	1.5
Spikelet density (mean distance (mm) between spikelets within a row)	2.2	3.5	3.3
Pollen stainability (%)	96	1	45
Fertility (%)	55.6	0	43.4

Seed was germinated following storage to overcome dormancy and a proportion of seedlings were albino, further suggesting the accession was sexual and also

suggesting it was diploid. The chromosome number was subsequently confirmed as 2n = 18 by examination of root-tip mitosis.

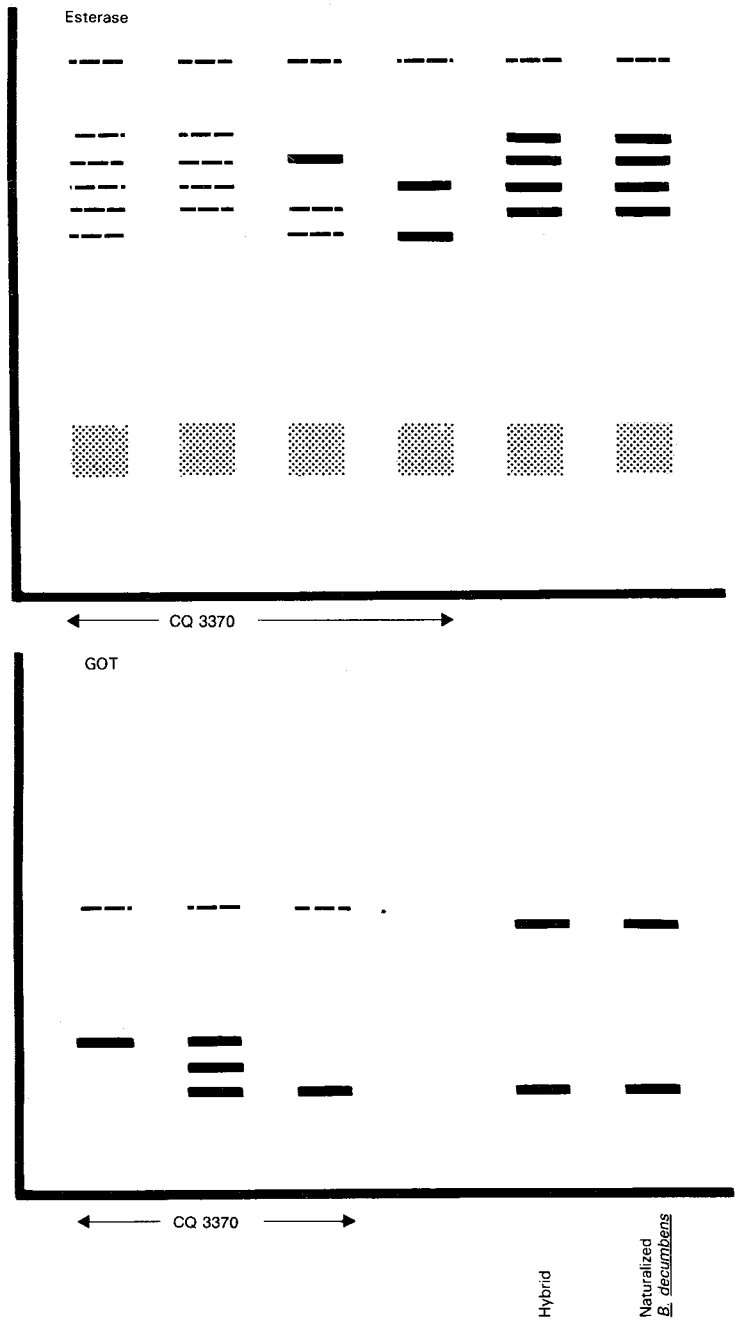

FIGURE 1

Esterase and GOT isozymes of CQ 3370, exhibiting genetic variation, and of a naturalized population of *Brachiaria decumbens* and their hybrid.

Cuttings from the 11 plants grown at Lawes together with 15 open-pollinated seedlings were grown in a glasshouse and assayed for variation using horizontal starch gel electrophoresis (Hayward and McAdam 1977). Gels were stained for glutamate-oxaloacetate transaminase (GOT), phosphoglucoisomerase (PGI), phosphoglucomutase (PGM), esterase, malate dehydrogenase (MDH), and reduced nicotinamide-adenine dinucleotide lipoamide oxidoreductase (NADH lipoamide oxidoreductase). Variation was clearly evident in GOT, in which single banded slow and fast homozygotes and triple-banded heterozygotes were identified (Fig. 1). The fast homozygote appeared in the progeny but not amongst the original 11 plants. Esterase isozymes exhibited 1-5 banded genotypes at Rf 0.59-0.86, probably indicating homozygosity or heterozygosity for at least 2 genes (Fig. 1). Gels stained for other isozymes provided no evidence of variation. The evidence for variation in isozyme banding, especially the appearance of novel variation in the progeny, provided strong evidence for sexuality in this accession.

Hybridization with B. decumbens

An attempt was made to hybridize CQ 3370 with naturalized populations of *B. decumbens* growing in the Brisbane area. These naturalized populations are morphologically similar to the commercial cultivar Basilisk and isozyme studies also suggested that they are of the same genotype as the cultivar. This material was used as it provided a ready and reliable source of pollen.

A single plant of CQ 3370 was grown in a glasshouse in isolation from other *Brachiaria* spp. On flowering, freshly cut panicles of naturalized *B. decumbens* collected early morning after anther emergence but before pollen shedding, were shaken over the expanded stigmas of CQ 3370 as pollen shedding occurred and then stood in water in close proximity to increase opportunity for pollination. This procedure was repeated at 1-2 day intervals while flowering of the CQ 3370 plant continued.

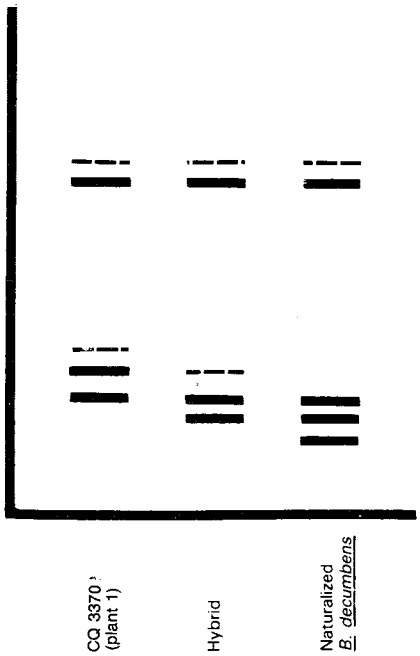

FIGURE 2
PGI isozymes of the *B. decumbens* hybrid and its 2 parents.

Two seeds were obtained, one of which germinated following removal of the enclosing palea and lemma. In vegetative growth emerging leaves were examined electrophoretically for signs of hybridity. GOT exhibited a single band in the slow region, similar to both parents. However, PGI exhibited a pattern in the hybrid intermediate between that of the 2 parents (Fig. 2). The slow band in the hybrid was present in the putative male parent but absent in the female parent and in all 22 plants studied in that accession. Esterase in the hybrid was similar to the male parent (Fig. 1) but this banding pattern also occurred in plants of CQ 3370.

Meiotic chromosome pairing was examined after the putative hybrid had been grown to maturity. Morphological comparisons were made in field-grown plants, and attempts were made to hybridize it in the field with the *B. decumbens* parent by open pollination.

Meiotic studies showed the plant to be a triploid, with $2n = 3x = 27$. Univalent frequency at first division metaphase and anaphase ranged from 6-9 (mean 7.3). Occasional cells were seen with trivalents, with up to 2 in a cell. The meiotic data confirmed hybridity and indicated homology of the diploid genome with 1 genome of the tetraploid parent, probably with 1-2 translocations. Attempts to produce a triploid by crossing *B. ruziziensis* with tetraploid *B. decumbens* have been unsuccessful (Ferguson 1974, cited by Gobbe *et al.* 1983) and the triploid identified in this study appears to be the first such record in the genus.

Morphological comparison of inflorescences with those of the 2 parents showed close similarity to the male parent (Table 1). The growth habit of the hybrid was slightly more decumbent than the male parent but it lacked the extended decumbent stems or stolons of CQ 3370. Pollen stainability was negligible and female fertility was zero in the presence of ample quantities of pollen from naturalized *B. decumbens.*

The infertility of the hybrid prevents it from being utilized directly in a breeding program. However, seed set may be possible if chromosome number of the hybrid were doubled using colchicine, or if a hybrid was made using autotetraploid CQ 3370 as has successfully been achieved with diploid *B. ruziziensis* (Gobbe *et al.* 1983). So far, attempts to double chromosome numbers in these *Brachiaria* genotypes have not been successful. Whether or not CQ 3370 represents a novel source of sexuality in *Brachiaria* depends on its relationship to *B. ruziziensis*, which, according to Clayton and Renvoize (1982) 'seems to be a local segregate from *B. decumbens*' (sic). However, the successful production of a triploid in this study suggests that CQ 3370 is more closely related to *B. decumbens* than is *B. ruziziensis.*

ACKNOWLEDGMENTS

I am grateful to Mr B. Simon, Queensland Herbarium for plant identification and to Messrs P. G. Stanton and D. Blogg for technical assistance.

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(Received for publication October 12, 1987; Accepted February 26, 1988)

CORRECTIONS TO VOLUME 22, NUMBER 2, JUNE 1988

Beef production from forage crops in the Brigalow region of central Queensland. 1. Forage sorghums—A. V. French, P. K. O'Rourke and D. G. Cameron (pages 79–84).

Page 82, Table 4

The units for the columns headed 'Average daily gain' should read (kg/hd), not (kg/ha/day).

Beef production from forage crops in the Brigalow region of central Queensland. 2. Winter forage crops—A. V. French, P. K. O'Rourke and D. G. Cameron (pages 85–90).

Page 89, Line 4

The text should read—
'4 start at a mean stocking rate of 2.1 beasts/ha giving 80 kg/hd liveweight gain (LWG)'.

Rotational and continuous grazing of Zulu forage sorghum (*Sorghum* spp. hybrid) by beef cattle grazed at 3 stocking rates—A. V. French, P. K. O'Rourke and D. G. Cameron (pages 91–93).

Page 93, Table 3

The units for the columns headed 'Average daily gain' should read (kg/hd) not (kg/ha).