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# Polyploidization of sexual diploid *Brachiaria decumbens* for intraspecific hybridization

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**Key words:** apomixis, chromosome duplication, forage breeding, polyploidization, signalgrass

**Introduction** *Brachiaria decumbens* (D), also known as signalgrass, is the most widely adapted forage grass in the tropics. It was first introduced to Brazil in the early 1950's and its best known cultivar, cv. Basilisk, quickly spread to the acid savannas of Latin America, covering millions of hectares. It is a natural tetraploid (4x) and apomictic genotype of good dry matter and seed production, even though it is susceptible to spittlebugs, the most widespread insect pest in tropical pastures. Breeding of this species was restricted to using 4x apomictic accessions as pollen donors in interspecific crosses, since sexual genotypes are diploid (2x). Earlier attempts of interploydic crosses were unsuccessful (Ferguson and Crowder, 1974; Hacker, 1994). This paper is the first report of the use of in vitro culture and colchicine treatments in tetraploidizing sexual genotypes of signalgrass.

**Material and methods** Plant meristems of several genotypes were isolated, inoculated in LS culture medium, and shoots obtained were transferred to MS medium for rooting. Basal segments were then separated and placed for 48 hours, on LS medium containing 0.01% colchicine. Surviving plants were later transferred to the greenhouse then to the field for evaluation. Chromosome countings were done both on root tips (mitosis) and in microspores (meiosis). Mode of reproduction was assessed using the Young et al., (1979) clearing technique and interference contrast microscopy.

**Results** From 1628 meristems extracted, 373 were treated with colchicine and 87 plants were recovered. The main problem was avoiding contamination in tissue culture since meristems were extracted from plants in pots. Until now, 21 were examined for ploidy level and cD24-2, cD24-27 and cD24-45 were confirmed as tetraploid either in mitosis or both meiosis and mitosis. Despite treating several accessions, duplication was achieved only in accession D24, more responsive than others to tissue culture and colchicine treatment. Duplicated plants maintained sexual behavior as determined by microscopic analysis of embryo sacs. Chromosome behavior on tetraploidized plants and pollen viability were assessed (Tables 1 and 2). Most chromosomes associated as bivalents but multivalents were also observed (Table 2). Several meiotic abnormalities were observed in varying frequencies, such as early migration to the poles, laggard chromosomes, and loss of DNA in micronuclei and microcytes.

**Table 1** *Pollen viability in induced tetraploid plants of* Brachiaria *and on apomictic genotype used for crosses.*

| Plant | % tetrads in meiosis | Viable (%) | Unviable (%) | Total grains analyzed |
|---|---|---|---|---|
| cD24-2 | 91.5% | 64.86 | 35.14 | 629 |
| cD24-27 | 97.6% | 61.36 | 38.64 | 1030 |
| cD24-45 | 94.6% | 63.25 | 36.75 | 928 |
| B. decumbens cv Basilisk | - | 73.82 | 26.12 | 1104 |

**Table 2** *Chromosome association in meiosis of induced tetraploid genotypes of* Brachiaria.

| Plants | Number of cells analyzed | Average chromosome association | | | |
|---|---|---|---|---|---|
| | | Ⅰ | Ⅱ | Ⅲ | Ⅳ, Ⅴ, Ⅵ |
| cD24-2 | 52 | 0.11 (0-2) | 16.92 (12-18) | 0.11 (0-2) | 0.42 (0-3) |
| cD24-27 | 30 | 0.77 (0-10) | 15.26 (8-18) | 0.5 (0-3) | 0.26 (0-3) |
| cD24-45 | 70 | 0.19 (0-2) | 16.2 (8-18) | 0.13 (0-2) | 0.76 (0-4) |

**Conclusions** Through many attempts, it was possible to obtain artificially tetraploidized sexual genotypes of signalgrass using tissue culture and colchicine. There were clear differences between genotypes in response to polyploidization. These are the first sexual compatible signalgrass genotypes, a novelty that will allow intraspecific hibridization with natural superior apomictic accessions in this important pasture species for the tropics.

**References**

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