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Use of RAPD markers to identify intraspecific hybrids of *Brachiaria humidicola*

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Introduction *Brachiaria humidicola*, also known as koronivia grass, is an African species well adapted to acid and poorly drained soils. It covers millions of hectares of pastures in tropical Latin America and it is used both for cattle and horses. Breeding was done for the first time in this species at Embrapa Beef Cattle, once a sexual tetraploid ecotype was identified in a collection of 58 accessions of *B. humidicola* introduced from eastern Africa. Controlled pollination in the greenhouse yielded hybrids from a cross between the sexual genotype and an apomictic accession. Since no emasculation was done, identification of hybrids vs. selfs became crucial thus RAPD markers were developed to discriminate hybrids precisely and early in the program.

Material and methods Hybridization between the sexual tetraploid accession H31 of *B. humidicola* and apomictic cultivar BRS Tupi (H16) produced 348 plants. Seeds were collected on the sexual plant only. DNA was extracted from leaves using the protocol established by Bonato et al. (2002). Ten randomic primers were selected based on discrimination between the two progenitors. The selection criterion was the presence of informative bands, i.e., those present in the apomictic parent and absent in the female parent. The identification of hybrids was done directly on the agarose gels analyzed. The presence of at least three informative bands characterized a hybrid genotype.

Results The ten selected primers amplified 31 informative markers. Primer AB01 was the one that amplifies most bands (Table 1). Among the plants analyzed, 285 presented bands from the apomictic parent (red arrows), which confirms their hybrid nature whereas 63 (18.1%) did not present any of those bands and were considered results from self-pollination. These can be discarded from the breeding program while the others are being phenotyped for mode of reproduction to study inheritance of apomixis in *B. humidicola*. Figure 1 exemplifies the RAPD profiles generated with primer AB01 in the parental material (Apo H16 and Sex H31) and in 17 plants from the population.

Table 1 *Sequence of nucleotides (SN) of the primers used and the number of informative markers (IM) amplified per primer.*

Primer	SN	IM	Primer	SN	IM
P14	CCAGCCGAAC	5	AC17	CCTGGAGCTT	2
81	GGAGCGTACT	3	AD01	CAAAGGCCGG	3
AB01	CCGTCGGTAG	6	AK04	AGGGTCGGTC	2
BA02	TGCTCGGCTC	1	AK19	TCGCAGCGAG	5
AB02	GGAAACCCCT	1	AL03	CCCACCCTTG	3

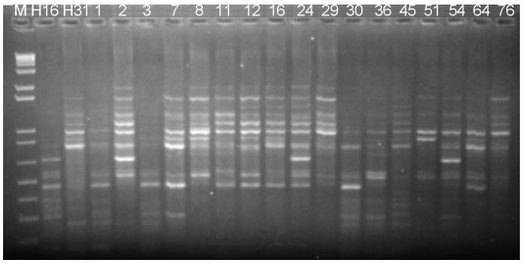

Figure 1. *RAPD profile of the parental material and 17 plants from the population under study using the AB01 primer.*

Conclusions RAPD markers adequately selected allow for early identification of hybrids. The approximate proportion of hybrid plants to those resulting from selfing was 5:1. *B. humidicola*, and allogamous plant, withstands selfing when subjected to artificial pollination in the greenhouse.

Reference

Bonato, A.L.V., Valle, C.B., Jank, L., Resende, R.M.S., Leguizámon, G.O.C. 2002. Extração de DNA genômico de *Brachiaria* e *Panicum maximum*. Campo Grande, MS: Embrapa Gado de Corte. (Embrapa Gado de Corte. Comunicado Técnico, 79).