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Short Communication

Identification of a molecular marker linked to apomixis in *Brachiaria humidicola* (Poaceae)

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With 1 figure and 1 table

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Abstract

A bulked segregant analysis using RAPD technique was carried out to identify molecular markers linked to apomixis in a *Brachiaria humidicola* F_1 population that segregated 1 : 1 for the mode of reproduction (apomixis and sexual). A marker related to the apo-locus was found. Segregation data, together with this marker were used to generate a map of the region. This marker was located at 4.61 cM of the target locus, and it can be used in deploying marker-assisted selection for mode of reproduction in the hybrid progenies of this species.

Key words: apospory — linkage map — RAPD — tropical forage grass

Brazil is the world's largest beef exporter and also has the biggest cattle herd, numbering about 200 million heads. This large herd feeds almost exclusively on pastures. Cultivated pastures in Brazil cover an estimated 120 million ha, and about 85% of this area is planted to *Brachiaria* species (Macedo 2006).

This paper describes the work carried out with a hybrid population of *B. humidicola* (Rendle) Schweickert, also known as koronivia grass, a perennial savanna grass native to Africa. This species is well adapted to poorly drained and infertile acid soils (Keller-Grein et al. 1996). Most *B. humidicola* ecotypes are polyploid and reproduce by apomixis, an asexual mode of reproduction through seeds (do Valle and Savidan 1996).

Apomixis in *B. humidicola* is facultative, pseudogamous and embryo sacs show apospory of the *Panicum* type, where the apospore develops into a diploid, monopolar four-nucleate embryo sac with a three-celled egg apparatus and a central cell with one nucleus (Savidan 1982). Pseudogamy means that for endosperm development the secondary nucleus of the embryo sac needs to be fertilized by a male gamete (Alves et al. 2001). Facultative apomictic plants develop both aposporous and meiotic sacs within the same inflorescence and even within the same pistil (Nogler 1984). The two reproductive modes are not mutually exclusive, therefore, some segregation may be observed in the progeny of an apomictic *Brachiaria* genitor. In the main *Brachiaria* species apomixis is simply inherited (do Valle and Savidan 1996), thus apomictic and sexual hybrids are generated at each sexual × apomictic cross in the proportion of 1 : 1.

The objective of this work was to identify RAPD markers tightly linked to apomixis in *B. humidicola* hybrids to improve efficiency and accuracy in the determination of the reproduction mode on the breeding programme of this species.

Materials and Methods

Plant material: One hundred and seven plants belonging to an F_1 population obtained by crossing a polyploid apomictic cultivar (*B. humidicola* cv. BRS Tupi) with a polyploid sexual parent (BRA005811-H31) were analysed. The hybrid nature of the F_1 plants was confirmed using RAPD markers.

Analysis of mode of reproduction: Flowers of these hybrids were collected at anthesis. Sixty ovules were dissected from each hybrid and then clarified using methyl salicylate according to the Young et al. (1979) procedure. At least 50 cleared ovules were analysed per plant using an interference contrast microscope. A chi-squared test was performed on this progeny to verify whether the genetic segregation for apomictic to sexual plants fit the expected model for a tetrasomic monogenic inheritance.

Molecular analysis: The DNAs were extracted from young leaves using the Bonato et al. (2002) method. The polymerase chain reactions (PCRs) and thermal conditions were performed according to the Bonato et al. (2002) procedure.

A bulked segregant analysis (Michelmore et al. 1991) using 270 RAPD primers was carried out to identify RAPD markers linked to apomixis. Five apomictic bulks (AB) and five sexual bulks (SB) were prepared, each one containing equimolar quantities of DNA from 10 phenotyped plants. Some plants were not used on the bulks, but were individually analysed.

Linkage analysis: Linkage analysis was conducted with the GQMOL computer software (Cruz and Schuster 2007) and a map was constructed. The LOD score used was 3.0. Kosambi's mapping function was used to convert recombination units into genetic distances (Kosambi 1944). The goodness of fit of segregation was tested by the chi-squared test.

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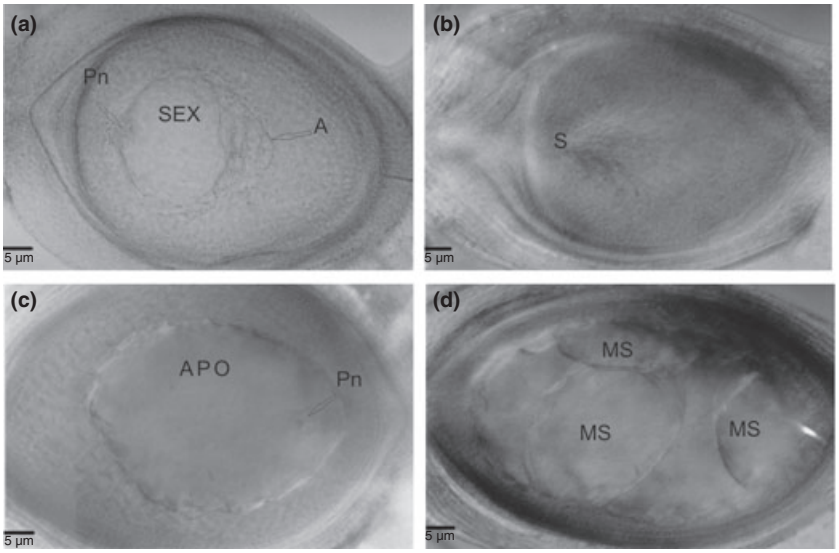

Fig. 1: Embryo sacs from *Brachiaria humidicola* hybrids observed under interference contrast microscopy. (A) Meiotic embryo sac – SEX from a sexual hybrid containing two polar nuclei – Pn and several multinucleated antipodal cells – A. (B) Sterile embryo sac – S. (C) Apomictic embryo sac – APO with only one polar nucleus and absence of antipodal cells. (D) Multiple apomictic embryo sacs – MS

Selection efficiency: Selection efficiency (SE) of the markers linked to the apomixis locus was based on the comparison between the phenotypic and the genotypic (markers) evaluations and was calculated according to Silva et al. (2007).

Results and Discussion

The *Panicum* type of aposporous embryo sac can be differentiated from the *Polygonum* (meiotic) type by the absence of antipodal cells and the presence of only one polar nucleus upon examination with interference contrast microscopy. Besides, aposporous sacs are rarely alone. The most common is to find two to three aposporous sacs within one ovule of the apomictic plant. The sexual plant will display only one *Polygonum* type sac (meiotic, or reduced sac) containing the egg cell, two polar nuclei and several large, multinucleated antipodal cells (Fig. 1). The proportion of meiotic and apomictic embryo sacs is given in Table 1.

Determination of mode of reproduction done on the 107 hybrids resulted in a proportion of apomictic to sexual plants not significantly different from the 1 : 1 ratio (P = 0.7718) with 55 apomictics plants and 52 sexual plants. The results corroborate the proposed hypothesis of a single dominant gene controlling apomixis in this hybrid population (do Valle and Savidan 1996).

Of the 217 RAPD primers tested on the parents, 32 (14.75%) amplified poorly or did not amplify. Of the remaining 185 primers, 176 (81.10%) were polymorphic on the parents and only seven (3.98%) amplified polymorphic markers between contrasting bulks.

Table 1: Variation on the percentage of the types of embryo sacs found in *Brachiaria humidicola* hybrids

		Types of embryo sacs (%)			
Reproduction mode	Number of plants	Meiotic	Aposporous (single and/or multiple)	Sterile	Abnormal
Sexual	55	23–100	0	0–64	0–66
Apomictic	52	0–65	18–91	0–28	0–70
Total	107				

Only one molecular marker of 650 bp obtained by amplification with the primer 64, named 64_650, resulted in a good fit considering a 1 : 1 segregation ratio (P = 0.4986) in the F_1 population and the linkage analysis revealed that this marker co-segregated with apomixis in *B. humidicola*. The distance between the 64_650 marker and the apomixis locus (apo-locus) was 4.61 cM and the value of LOD score was 23.44. Five recombinant individuals were found between the 64_650 marker and the apomixis locus. The assisted selection efficiency for this marker linked to the apo-locus was 95.6%.

Although, apomixis *in B. humidicola* does not exhibit complete penetrance, as observed by the percentage of meiotic embryos in apomictic plants (Table 1), the possibility of incomplete penetrance was not included in the molecular marker analysis. The number of embryos analysed for each F_1 plant enabled a precise identification of its mode of reproduction. The molecular marker identified in this work may be used for assisted selection of the apomixis trait; however, it is important to consider that meiotic embryos may be produced by apomitic plants.

The use of this molecular marker for detection of apomixis in *B. humidicola* can certainly increase the ability to accurately classify progenies. This technology offers the prospect of rapid and early screening for the trait. Furthermore, the identification and characterization of the genomic regions involved in the phenomenon of apomixis in the different species where the trait has been described is an initial and essential step in the process of introgression of this trait into valuable crops.

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