Construction of Microsatellite-Enriched Libraries for Tropical Forage Species and Characterization of the Repetitive Sequences Found in *Brachiaria Brizantha*

Letícia Jungmann
Universidade Esdatual de Campinas, Brazil

C. B. do Valle
EMBRAPA, Brazil

P. R. Laborda
Universidade Esdatual de Campinas, Brazil

Rosangela M. S. Resende
EMBRAPA, Brazil

Liana Jank
EMBRAPA, Brazil

The XX International Grassland Congress took place in Ireland and the UK in June-July 2005. The main congress took place in Dublin from 26 June to 1 July and was followed by post congress satellite workshops in Aberystwyth, Belfast, Cork, Glasgow and Oxford. The meeting was hosted by the Irish Grassland Association and the British Grassland Society.

Proceedings Editor: D. A. McGilloway

Publisher: Wageningen Academic Publishers, The Netherlands

Presenter Information
Letícia Jungmann, C. B. do Valle, P. R. Laborda, Rosangela M. S. Resende, Liana Jank, and A. P. de Souza

Construction of microsatellite-enriched libraries for tropical forage species and characterization of the repetitive sequences found in *Brachiaria brizantha*

L. Jungmann[1,2], C.B. do Valle[1], P.R. Laborda[2], R.M.S. Resende[1], L. Jank[1] and A.P. de Souza[2]
[1]*Plant Biotechnology Laboratory, Embrapa Beef Cattle, CP 154, CEP 79.002-970. Campo Grande, MS, Brazil*
[2]*CBMEG, Universidade Esdatual de Campinas, CP 6010, CEP 13.083-875, Campinas, SP, Brazil*
E-mail: jungmann@cnpgc.embrapa.br

Keywords: tropical forages, molecular markers, microsatellites, breeding program

Introduction The Brazilian cattle herd comprises 185 million animals fed with about 177 million hectares of native and cultivated pastures (IBGE, 2002). Of the grass species used for forage in Brazil, the African genus *Brachiaria* is the most widely planted, followed by *Panicum*, which also has an African origin. Legumes of the *Stylosanthes* genus, native to the South America, have emerged in the last few years as potential forage species for use with the grasses. These forage species have been bred at Embrapa Beef Cattle and the breeding programs have shown the need for more genetic information including the use of molecular markers. The objectives of this work were to construct microsatellite-enriched genomic libraries for 5 species of *Brachiaria* (*B. brizantha, B. decumbens, B. dictioneura, B. humidicola and B. ruziziensis*), for *P. maximum* and for *S. capitata*, and to characterize the microsatellites found in *B. brizantha*.

Materials and methods One genotype from the germplasm collection of EMBRAPA for each species mentioned above was used to develop libraries enriched for $(AG)_n$ and $(AC)_n$ dinucleotides. Construction of genomic libraries was carried out as described previously by Billotte *et al.* (1999). Basically this involves (1) genomic DNA digestion with a restriction enzyme, (2) ligation of oligonucleotide adaptors to fragments, followed by PRC amplification, (3) hybridization of microsatellite containing fragments with $(CT)_8$ and $(GT)_8$ biotinylated oligonucleotides, (4) recovery of hybridized fragments with streptavidin coated magnetic beads, followed by PCR amplification and (5) cloning of amplified fragments in the pGEM-T vector. The presence of microsatellite containing fragments in the library was confirmed after sequencing 20 clones from each library. Sequences were analyzed using the Simple Sequence Repeat Identification Tool-SSRIT (Temnykh *et al.*, 2001) to identify those containing simple perfect repetitive motifs.

Results A total of seven libraries enriched for both AC and AG were constructed, one for each species. Libraries had an average insert size of 700 bp. Sequencing of 20 clones per library showed that about 80% had repetitive motifs. Analysis of 212 *B. brizantha* clones carried out using the SSRIT (which searches only for simple and perfect motifs) found 360 repetitive motifs, 298 (82.7%) being dinucleotides and 62 (17.22%) being tri, tetra and pentanucleotides. No hexanucleotides containing perfect motifs was found. Figure 1 shows the frequency of the dinucleotides motifs. As expected due to the enrichment procedure, AC/GT, CA/TG, AG/CT and GA/TC were the most frequent motifs found. Searches for compound and/or imperfect motifs were not carried out. From all the simple and perfect motifs found, only 13 could be considered as microsatellites, based on the number of repetitions of the motifs. From these, 9 were located in regions appropriate for microsatellite amplification primer design.

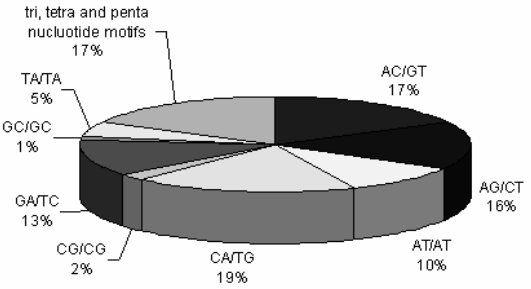

Figure 1 Frequency of repetitive motifs in the *B. brizantha* genome

Conclusions The results demonstrate that SSR enriched libraries for 5 species of *Brachiaria*, and for *Panicum maximum* and *Stylosanthes capitata* can be obtained. Analysis of *B. brizantha* repetitive motifs showed that the most common dinucleotide motifs were AC/GT and AG/CT. From all the repetitive motifs found, only 9 could be used for microsatellite primer design which means that more clones should be sequenced and analyzed.

References

Billotte N, Lagoda PJ, Risterucci A-M & Baurens F-C (1999). Microsatellite-enriched libraries: applied methodology for the development of SSR markers in tropical crops. Fruits 54:277-288.

IBGE (Geography and Statistics Brazilian Institute) (2002) Censo agropecuário. www.ibge.gov.br

Temnykh S, DeClerck G, Lukashova A, Lipovich L, Cartinhour S & McCouch S (2001). Computational and experimental analysis of microsatellites in rice (Oryza sativa L.): frequency, length variation, transposon associations, and genetic marker potential. Genome Research 11:1441-1452.