WORKING GROUP ON BIOCHEMICAL AND MOLECULAR TECHNIQUES, AND DNA-PROFILING IN PARTICULAR

Thirteenth Session
Brasilia, November 22 to 24, 2011

ADDENDUM

EVALUATION OF A GERmplASM COLLECTION OF BRACHIARIA HUMIDICOLA USING MICROSATELLITES, MORPHOLOGICAL MARKERS, CYTOGENETICS AND GEOGRAPHICAL ORIGIN

Documents prepared by experts from Brazil
Evaluation of a germplasm collection of *Brachiaria humidicola* using microsatellites, morphological markers, cytogenetics and geographical origin

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Pasture Areas in Brazil

Adapted from Brazilian Institute for Geography and Statistics (IBGE), 2005

TOTAL CERRADO AREA

NATIVE PASTURES:
1 Cerrado of Roraima
2 Savanna of Amapá
3 Marajó Island
4 Baixo Maranhão
5 Semi-arid region
6 Pantanal
7 Pampa Gaúcho
8 Baixo Amazonas

170 millions ha
70% Cultivated

Cattle distribution in Brazil: density

Source: IBGE, Censo Agropecuário 2006
2nd half of 20th Century:
Expansion of cattle activities in Brazil
Introduction of African species in Brazil, to be used as forage

Source: Brazilian Institute for Geography and Statistics (IBGE)
World Beef Trade Flows

Source: Global Trade Atlas database (GTIS)
Germplasm collection of *Brachiaria* at Embrapa Beef Cattle
## Ploidy level and citogenetics

<table>
<thead>
<tr>
<th>SPECIES</th>
<th>N. of accessions</th>
<th>Ploidy</th>
<th>2x</th>
<th>4x</th>
<th>5x</th>
<th>6x</th>
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<td>2x</td>
<td>4x</td>
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<td>157</td>
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<td>47+2</td>
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</table>

### SSR development for tropical forages:

**Grasses:**


**Legumes:**

- *Calopogonium mucunoides* – Sousa et al. Molecular Ecology Resources 2010 (accepted)
**Bracharia humidicola (Rendle) Schweick.**

Koronivia grass; creeping signal grass; false creeping paspalum; braquiaria dulce; kikuyu de la Amazonia, pasto humidicola

Adopted as pasture in infertile and poorly drained soils or with seasonal flooding

Basic chromosome number: x=6 → tetra, hexa and nonaploids

57 apomicts + 1 sexual accession at EBC

Source: www.tropicalforages.info

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**Genetic diversity and population structure analysis of the tropical pasture grass Bracharia humidicola based on microsatellites, cytogenetics, morphological traits, and geographical origin**


Bracharia humidicola (Rendle) Schweick. is a warm-season grass currently used as pasture in the tropics. Accessions of this species were collected in eastern Acrea and northern humidicola in the 1980s. Bracharia humidicola is a geographically diverse species, with limited knowledge of its genetic diversity. This genetic diversity has prompted interest in understanding the patterns of genetic diversity within the species. In this study, we used microsatellite markers to investigate the genetic diversity of the species. The results indicate that the genetic diversity of Bracharia humidicola is high, with a high level of genetic variation observed within and between accessions. The results also suggest that the genetic diversity of Bracharia humidicola is influenced by environmental factors, with the genetic diversity of accessions from different regions showing significant differences. These findings suggest that Bracharia humidicola is a species with high genetic diversity, which could be important for the conservation and utilization of the species. The results also highlight the importance of further research to understand the genetic diversity of Bracharia humidicola and to develop effective conservation strategies. 

Source: www.tropicalforages.info

**Germplasm Diversity Characterization with SSRs**

Comparison with:
1. Collection sites of the accessions
2. Reproductive mode and ploidy levels
3. Genetic diversity revealed by morphological traits

58 accessions and 2 cultivars of *B. humidicola* were genotyped with 27 microsatellite loci

- Allelic frequencies could not be estimated due to ploidy levels of individuals
- Genetic distances could not be calculated from molecular data because these parameter is estimated from allelic frequencies
- Jaccard's similarities were used instead
- Molecular data were also subjected to analyses using Bayesian statistics

Pritchard et al. (2000) discussed the suitability of using distance-based methods and model-based methods for dealing with genetic data of a sample of individuals.

These authors stressed the advantages of the fine statistical inference that can be made using model-based approaches and indicated how the comparison between these two types of results could be useful for obtaining biologically suitable clues about the genetic diversity of a data set.
Clusters formed using Bayesian statistics supported groups resulting from similarity matrices. The two methods applied to molecular data were complimentary.

Model-based results revealed information on genic pools that compose this plant material that could not be accessed with the similarity-based grouping methodology.

The sole sexual accession of this germplasm collection formed an outgroup in relation to the apomictics.

24 morphological traits were evaluated in *B. humidicola* accessions by Assis et al. (2003)

- **6 vegetative traits**: plant height, plant growth habit, leaf length, leaf width, leaf growth habit, and length of leaf sheath

- **10 reproductive traits**: length of floral stem, inflorescence length, number of racemes, length of basal raceme, number of spikelets on basal raceme, spikelet insertion, rachis width, spikelet spot percentage, stigma color, and anther color

- **8 pubescence traits**: density of rachis pubescence, length of rachis pubescence, density of leaf blade pubescence, length of leaf blade pubescence, margin of the leaf blade, density of leaf sheath pubescence, length of leaf sheath pubescence, and distribution of leaf sheath pubescence

Euclidean distances were calculated from morphological data.
No correlation was found between genetic distances calculated from morphological descriptors and genetic similarities calculated from microsatellite markers.

Available geographic coordinates of the accession collection sites were retrieved from the Forages Database (CIAT 2009) and a matrix of linear distances (metres) between pairs of genotypes was generated.

Number of chromosomes and predicted ploidy levels were previously reported by Boldrini (2009) and were considered in the analysis.
Thank you!!!

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