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BMT/13/16 Add.
ORIGINAL: English
DATE: December 8, 2011

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INTERNATIONAL UNION FOR THE PROTECTION OF NEW VARIETIES OF PLANTS
GENEVA

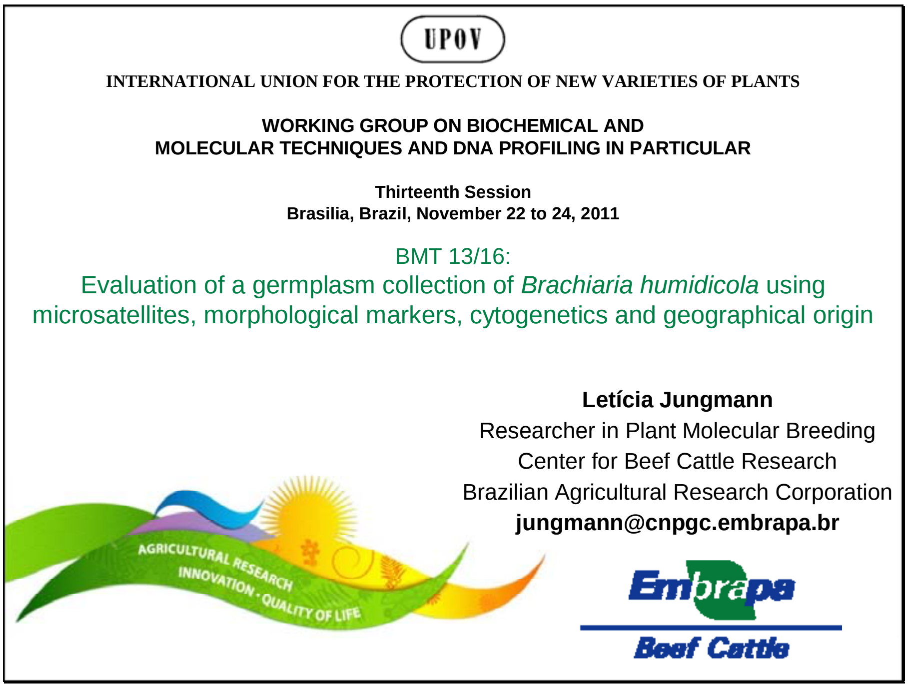
WORKING GROUP ON BIOCHEMICAL AND MOLECULAR TECHNIQUES, AND DNA-PROFILING IN PARTICULAR

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



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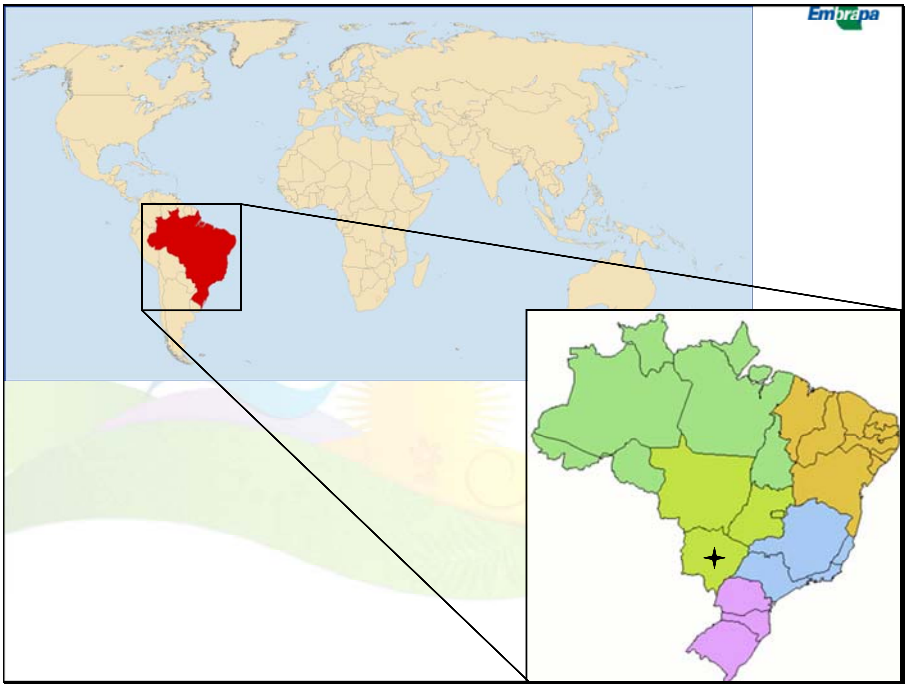
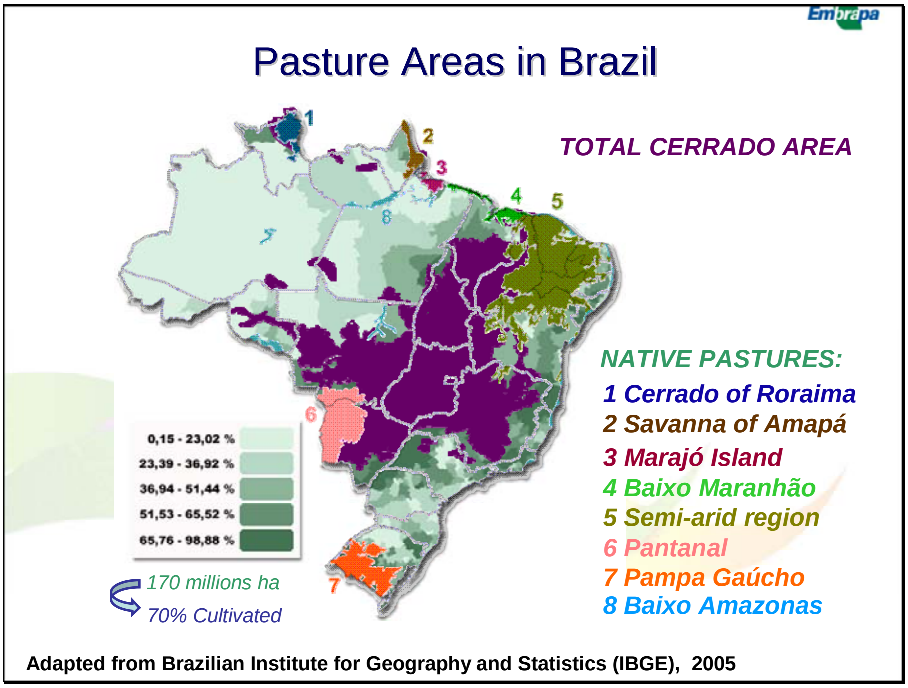
BMT 13/16:

Evaluation of a germplasm collection of *Brachiaria humidicola* using microsatellites, morphological markers, cytogenetics and geographical origin

Letícia Jungmann
Researcher in Plant Molecular Breeding
Center for Beef Cattle Research
Brazilian Agricultural Research Corporation
jungmann@cnpgc.embrapa.br





Pasture Areas in Brazil

TOTAL CERRADO AREA

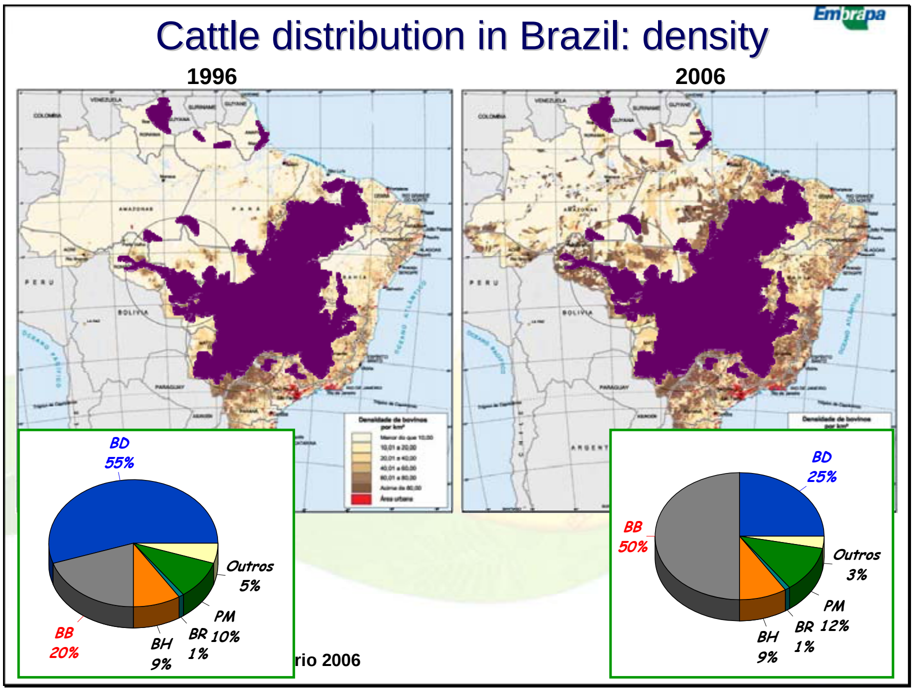
170 millions ha

70% Cultivated

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*

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

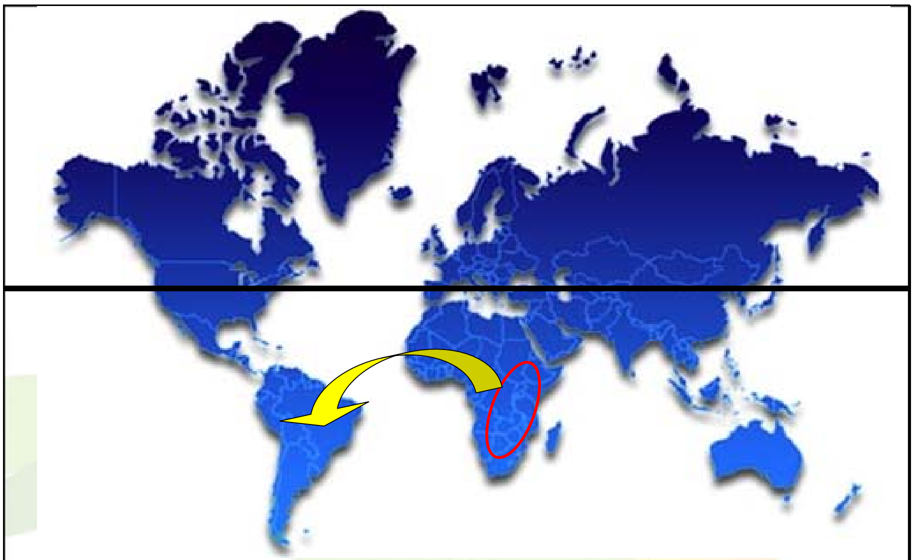


Cattle distribution in Brazil: density

1996

2006

rio 2006

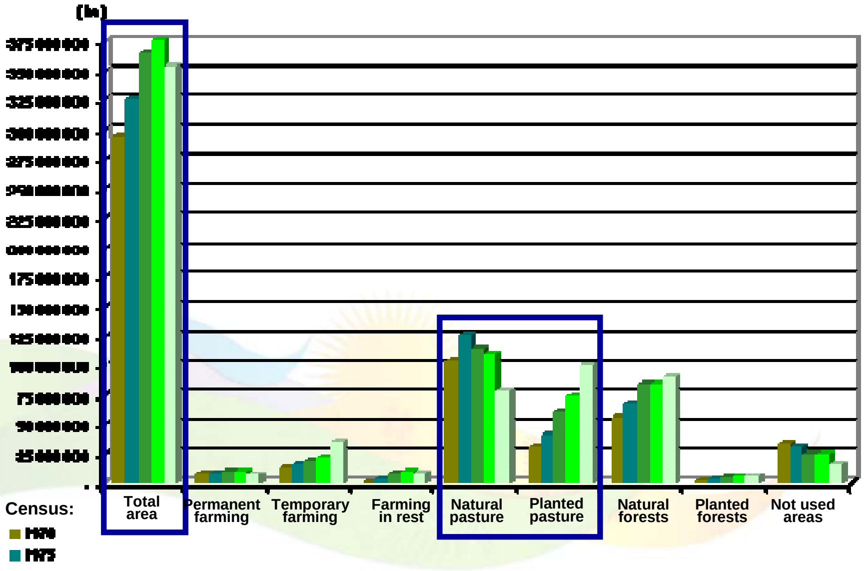


2nd half of 20th Century:

Expansion of cattle activities in Brazil

Introduction of African species in Brazil, to be used as forage

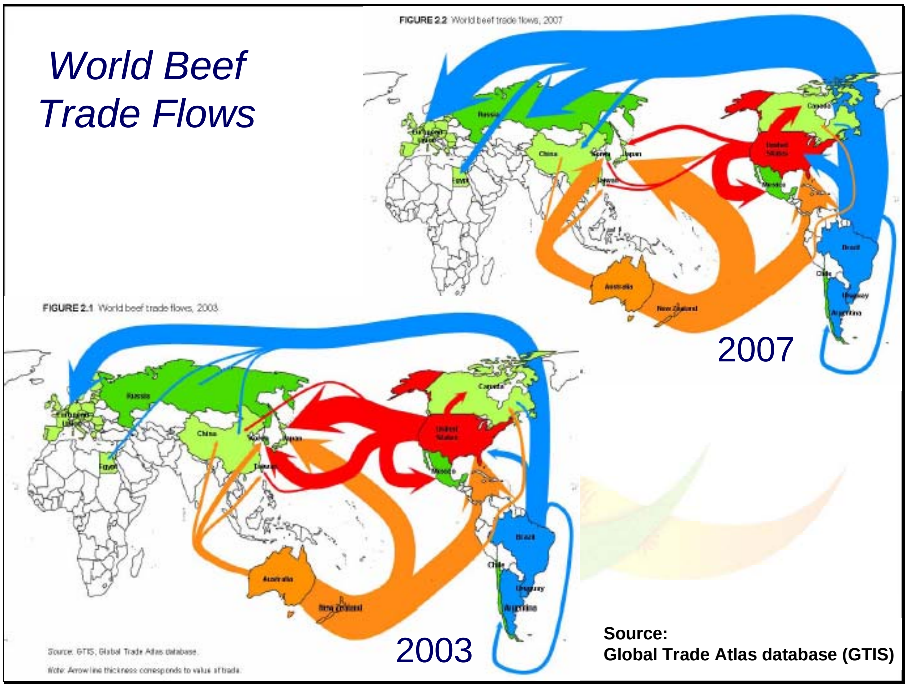
Disribution of activities in Brazilian lands



Source: Brazilian Institute for Geography and Statistics (IBGE)

(ha: 10,000 m²)


Embrapa



World Beef Trade Flows

FIGURE 2.2 World beef trade flows, 2007

2007

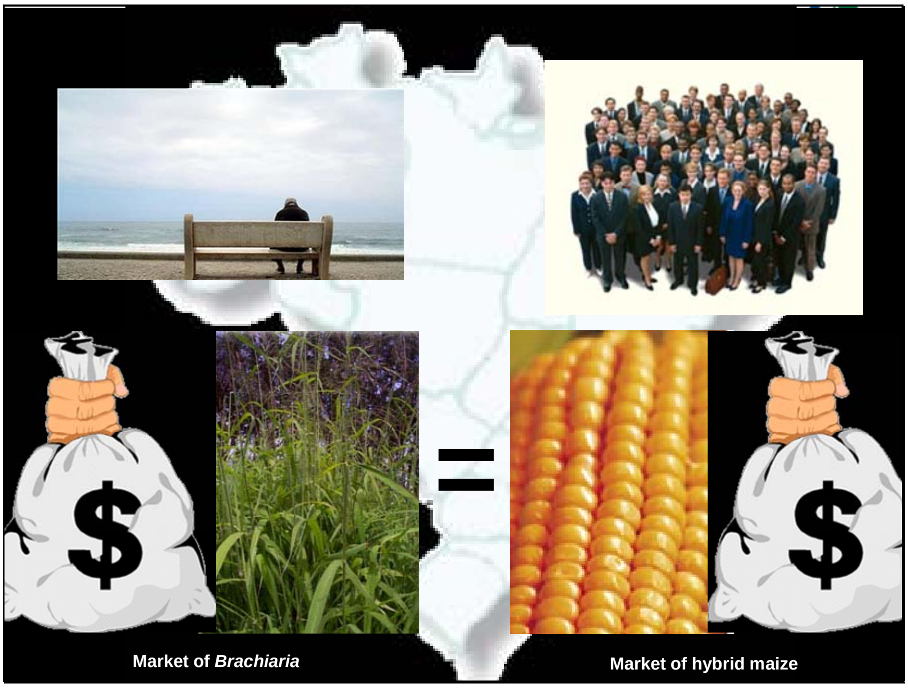
FIGURE 2.1 World beef trade flows, 2003

2003

Source: GTIS, Global Trade Atlas database.

Note: Arrow line thickness corresponds to value of trade.

Source:
Global Trade Atlas database (GTIS)



Market of *Brachiaria*

Market of hybrid maize

Germplasm collection of *Brachiaria* at Embrapa Beef Cattle

Ploidy level and citogenetics

SPECIES	N. of accessions in the collection	Ploidy			
		2x	4x	5x	6x
B. arrecta	5	-	5	-	-
B. brizantha	**222**	**2**	**157**	**41**	**22**
B. decumbens	**51**	**23**	**23**	**5**	**-**
B. dictyoneura	8	-	6	-	2
B. dura	2	-	-	2	-
B. eruciformis	1	1	-	-	-
B. humidicola	**60**	**-**	**22**	**18**	**19+1**
B. jubata	30	4	12	13	+1
B. leucacranta	1	-	-	1	-
B. nigropedata	21	-	19	-	2
B. mutica	*3*	-	*3*	-	-
B. platynota	2	2	-	-	-
B. ruziziensis	**29**	**24**	**5***	**-**	**-**
B. subulifolia	*2*	1	1	-	-
TOTAL	437	54	254	77	47+2

SSR development for tropical forages:

Grasses:

Brachiaria brizantha – Jungmann et al. Conservation Genetics 2009 DOI 10.1007/s10592-009-9839-7

Brachiaria humidicola – Jungmann et al. Conservation Genetics Resources 2009 DOI 10.1007/s12686-009-9111-y

Paspalum notatum – Cidade et al. Conservation Genetics 2009 DOI 10.1007/s10592-009-9872-6

Panicum maximum – Sousa et al. Plant Breeding 2010 DOI10.1111/j.1439-0523.2010.01779.x

Legumes:

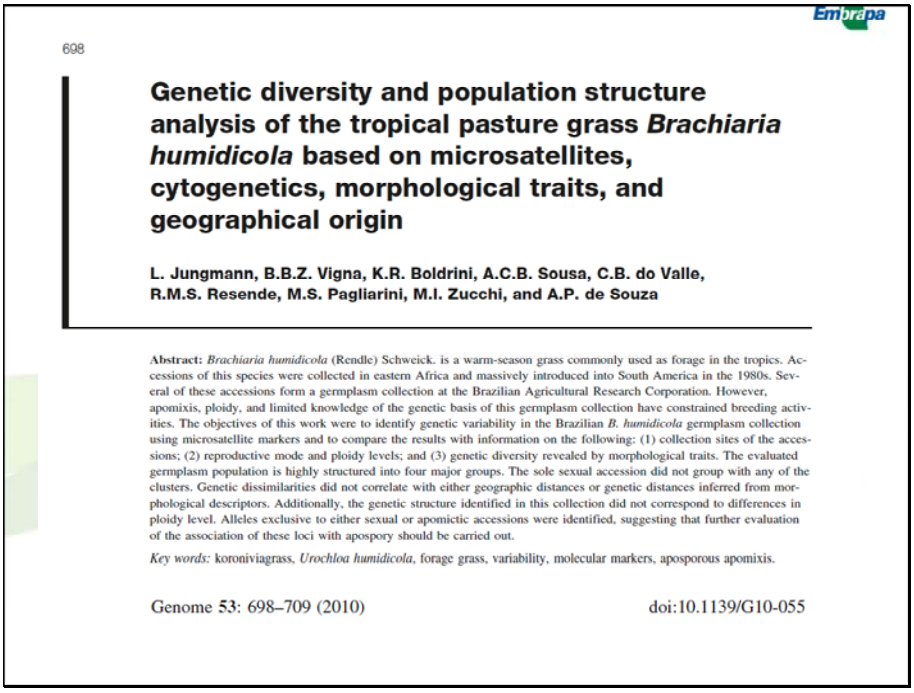
Centrosema pubescens – Sousa et al. Conservation Genetics Resources 2009 DOI 10.1007/s12686-009-9080-1

Calopogonium mucunoides – Sousa et al. Molecular Ecology Resources 2010 (accepted)

Stylosanthes guianensis – Santos et al. Conservation Genetics Resources 2009 DOI 10.1007/s12686-009-9010-2

Stylosanthes macrocephala – Santos et al. Conservation Genetics 2009 DOI 10.1007/s12686-009-9112-x

Stylosanthes capitata – Santos et al. Molecular Ecology Resources 2009 DOI 10.1111/j.1755-0998.2008.02308.x



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

L. Jungmann, B.B.Z. Vigna, K.R. Boldrini, A.C.B. Sousa, C.B. do Valle, R.M.S. Resende, M.S. Pagliarini, M.I. Zucchi, and A.P. de Souza

Abstract: *Brachiaria humidicola* (Rendle) Schweick. is a warm-season grass commonly used as forage in the tropics. Accessions of this species were collected in eastern Africa and massively introduced into South America in the 1980s. Several of these accessions form a germplasm collection at the Brazilian Agricultural Research Corporation. However, apomixis, ploidy, and limited knowledge of the genetic basis of this germplasm collection have constrained breeding activities. The objectives of this work were to identify genetic variability in the Brazilian *B. humidicola* germplasm collection using microsatellite markers and to compare the results with information on the following: (1) collection sites of the accessions; (2) reproductive mode and ploidy levels; and (3) genetic diversity revealed by morphological traits. The evaluated germplasm population is highly structured into four major groups. The sole sexual accession did not group with any of the clusters. Genetic dissimilarities did not correlate with either geographic distances or genetic distances inferred from morphological descriptors. Additionally, the genetic structure identified in this collection did not correspond to differences in ploidy level. Alleles exclusive to either sexual or apomictic accessions were identified, suggesting that further evaluation of the association of these loci with apospory should be carried out.

Key words: koroniviagrass, *Urochloa humidicola*, forage grass, variability, molecular markers, aposporous apomixis.

Genome 53: 698–709 (2010) doi:10.1139/G10-055

Brachiaria humidicola (Rendle) Schweick.

Source: www.tropicalforages.info

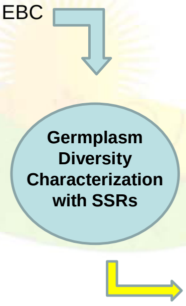
Koronivia grass; creeping signal grass; false creeping paspalum; braquiaria dulce; kikuyu de la Amazonía, pasto humidícola

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

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

57 apomictics + 1 sexual accession at EBC

C. B. Valle – Embrapa



Germplasm Diversity Characterization with SSRs

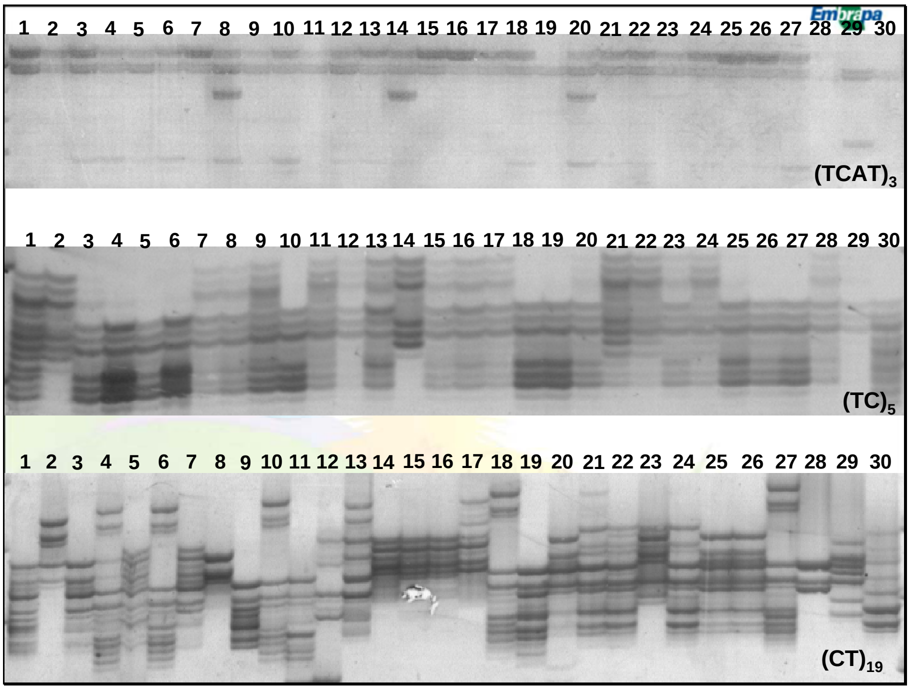
Comparison with:
(1)collection sites of the accessions;
(2) reproductive mode and ploidy levels;
(3) genetic diversity revealed by morphological traits.

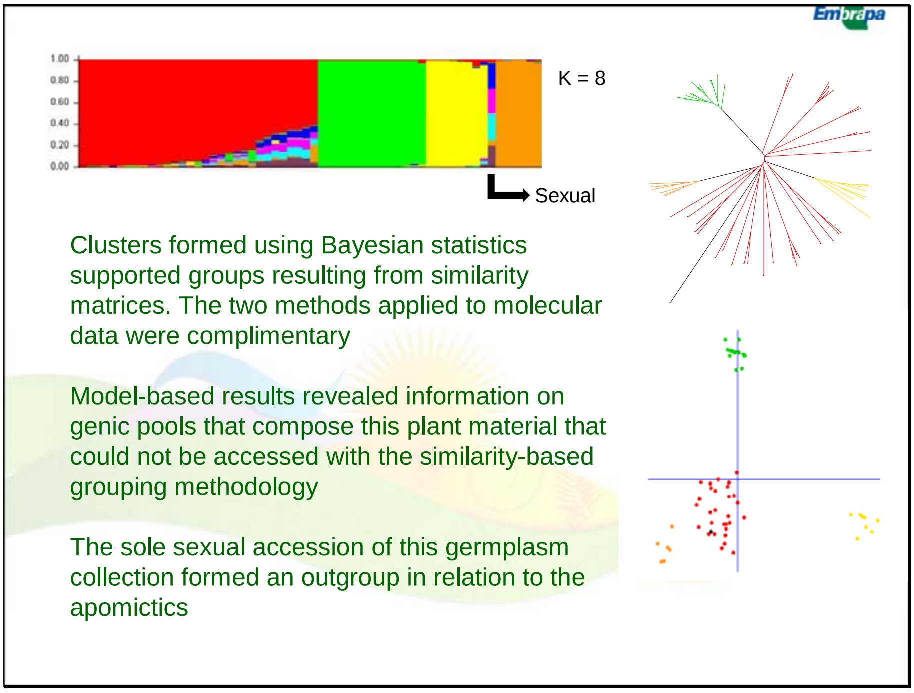
58 accessions and 2 cultivars of *B. humidicola* were genotyped with 27 microsatellite loci

- 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

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

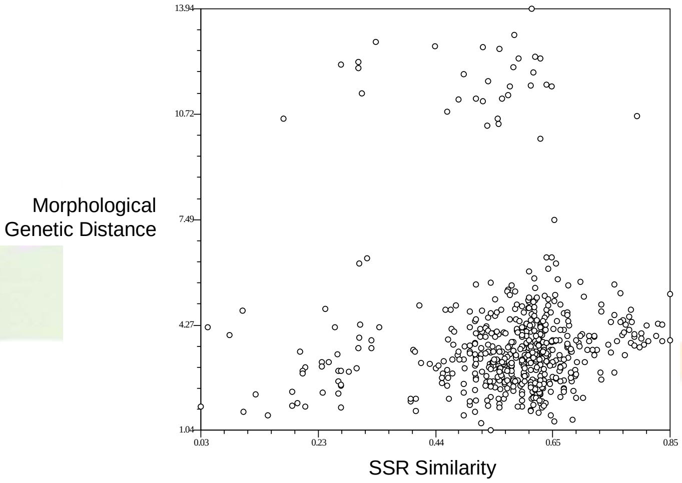
- **6 vegetative traits**: plant height, plant growth habit, leaf length, leaf width, leaf growth habit, and length of leaf sheath

- **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 ubescence, and distribution of leaf sheath pubescence

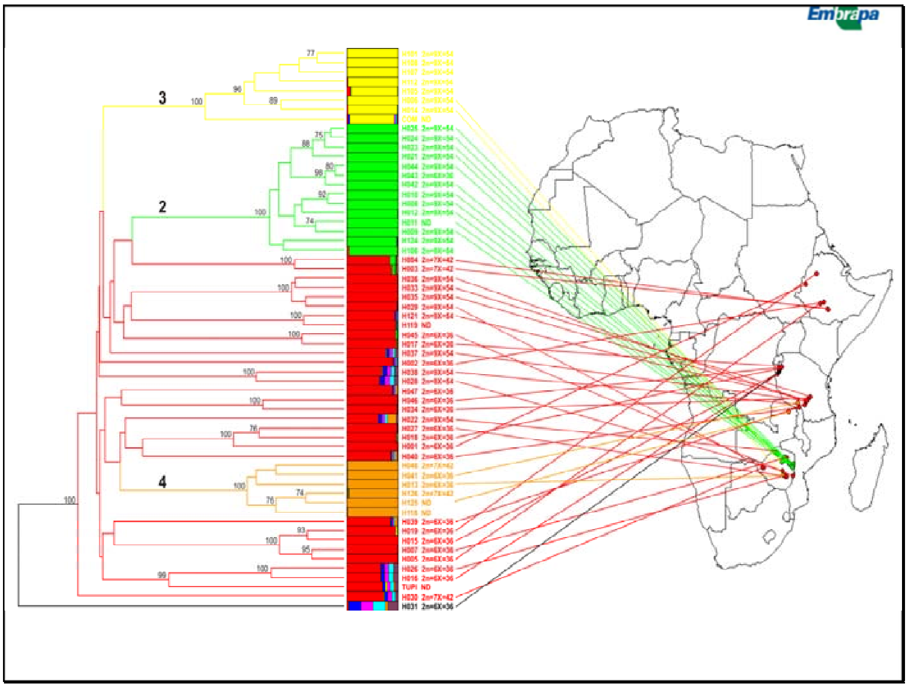
Euclidean distances were calculated from morphological data

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


3
2
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100
77
96
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75
88
80
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92
74
76
93
95
99
H101 2n=9X=54
H108 2n=9X=54
H107 2n=9X=54
H112 2n=9X=54
H105 2n=9X=54
H006 2n=9X=54
H014 2n=9X=54
COM ND
H025 2n=9X=54
H024 2n=9X=54
H023 2n=9X=54
H021 2n=9X=54
H044 2n=9X=54
H043 2n=6X=36
H042 2n=9X=54
H010 2n=9X=54
H008 2n=9X=54
H012 2n=9X=54
H011 ND
H009 2n=9X=54
H124 2n=9X=54
H106 2n=9X=54
H004 2n=7X=42
H003 2n=7X=42
H036 2n=9X=54
H033 2n=9X=54
H035 2n=9X=54
H029 2n=9X=54
H121 2n=9X=54
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H045 2n=6X=36
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H037 2n=9X=54
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H046 2n=6X=36
H034 2n=6X=36
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H027 2n=6X=36
H018 2n=6X=36
H001 2n=6X=36
H040 2n=6X=36
H048 2n=7X=42
H041 2n=6X=36
H013 2n=6X=36
H126 2n=7X=42
H125 ND
H118 ND
H039 2n=6X=36
H019 2n=6X=36
H015 2n=6X=36
H007 2n=6X=36
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H030 2n=7X=42
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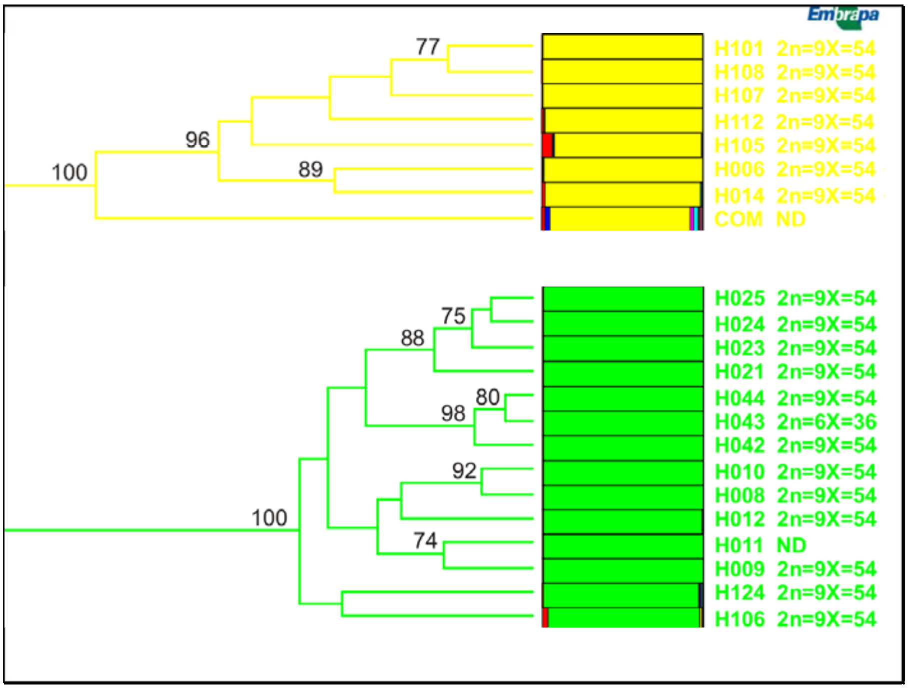
77
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74
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H108 2n=9X=54
H107 2n=9X=54
H112 2n=9X=54
H105 2n=9X=54
H006 2n=9X=54
H014 2n=9X=54
COM ND
H025 2n=9X=54
H024 2n=9X=54
H023 2n=9X=54
H021 2n=9X=54
H044 2n=9X=54
H043 2n=6X=36
H042 2n=9X=54
H010 2n=9X=54
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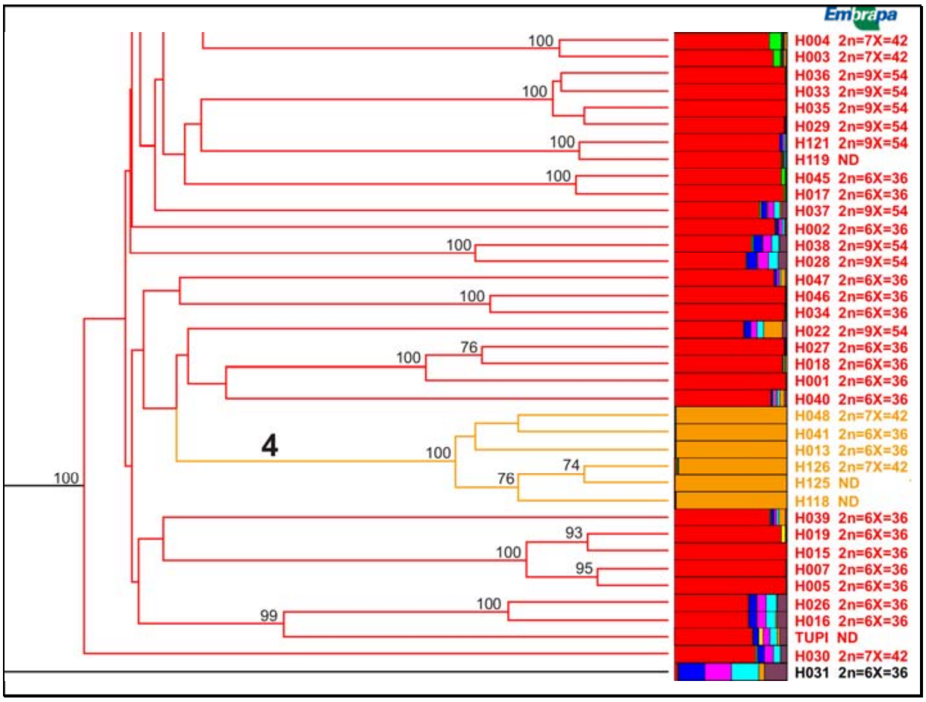
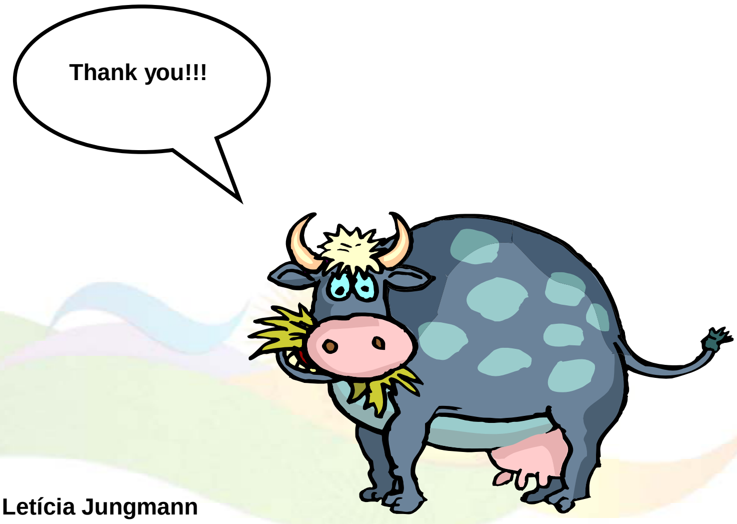
H011 ND
H009 2n=9X=54
H124 2n=9X=54
H106 2n=9X=54





Letícia Jungmann
jungmann@cnpgc.embrapa.br

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