LINKAGE MAP WITH ALLELE DOSAGE OF THE POLYPLOID *Megathyrsus maximus*

Candidate: Thamiris Gatti Deo
Supervisor: Dr. Anete Pereira de Souza
Co-supervisor: Dr. Liana Jank
412 million acres

Figure. Pastures in Brazil (yellow).
Guineagrass (syn. Panicum maximum)
- Most productive tropical forage
- High potential for biomass production (biofuel feedstock)

- Autotetraploid (base = 8 chromosomes; 2n = 4x = 32 chromosomes)

- Polyploidy and recent domestication → high genetic variability
Guineagrass
- Breeding program (40 years)
- Germplasm Collection (426 accesses)
- Significant annual increase in productivity per pasture area

Fonte: Liana Jank.
Introduction

- Sequenced genome
- Primary genetic information
- Apomixis
- Linkage map
- Non-model species
- Heritage
- Evolution genome
- Structure genome
- Traits
Introduction

**Apomixis**
- Asexual reproduction by seeds;

**Apospory-Specific Genomic Region (ASGR)**
- Dominant character
  - A single or few genes

**Markers intrinsically linked to ASGR**

**Distinguish sexual and apomictic plants**

- Progeny Tests
- Embryo sac analysis
SNP Markers and Linkage Mapping

- Single dose for one parent or both parents

\[(Aaaa \times aaaa)\]
\[(Aaaa \times Aaaa)\]

- Other doses \((AAaa, AAAa)\) lost

Maps of polyploids poorly saturated and informative

NGS

SNP multiple doses
Main goal

To construct a linkage map for *Megathyrsus maximus* and to map the apomixis region, improving the genetic knowledge of tropical forages grasses.
Methods

Sexual genotype tetraploidizada × Apomictic genotype ‘cv. BRS Mombaça’

136 F1 hybrids

Figure. Mapping population at Embrapa Beef Cattle.
**Methods**

**Extraction**

![Extraction Image](image)

**GBS library**

- **barcode**
- **PstI cut site**
- **DNA fragment**
- **MspI cut site**
- **common adapter**

**NGS Sequencing**

- **NextSeq 500**
- Single-end reads
- 150 bp reads
- 400 million reads
Data analysis

SNP calling
- TASSEL4-Poly – Modified for polyploid
- Alignment (Bowtie2) - Switchgrass (P. virgatum)

Methods

Sequencing quality
- NGS QC Toolkit

SNP calling

Linkage analysis
- TetraploidSNPMap

Filter quality
- Monomorphic markers
- Missing data 25%

Allele dosage
- Deph ≥ 60 reads
- Dosage (Updog)
Methods

Linkage map

Inferring Phases
- TetraploidSNPMAP

Cytoembryological analysis

Apomixis locus region

Apomixis locus region
- TetraploidSNPMAP
  QTL module
Alignment to Switchgrass genome

- 75.8% Aligned 0 times
- 24.2% Aligned exactly 1 time
- 7.4% 24,000 SNPs
- 24.2% 55,000 SNPs

Results and Discussion

Alignment

SNP calling

720 million reads of 138 individuals

Phred score 30

Fonte: Center for Crop Diversification
Results and Discussion

Allele depth
Allele dosage
Manual filtering

Depth ≥ 60 reads

SNP

23,619
2,529
1,322

Aligned only one time
Allele dosage
Filter quality

Manual filtering
Depth ≥ 60 reads
Missing data ≥ 25%

PC1 (4.9%)
PC2 (4.4%)
Results and Discussion

858 SNP markers with multiple dose
Size of 756.7 cM
Average density 1.13 cM
Greater distance – 8.6 cM - Group 5

Ebina et al. (2005) – 360 single dose markers AFLP and RAPD
## Results and Discussion

<table>
<thead>
<tr>
<th>HG</th>
<th>SNP</th>
<th>Size (cM)</th>
<th>Longest distance between markers (cM)</th>
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<tr>
<td>1</td>
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<td>101.7</td>
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<td>49</td>
<td>70.1</td>
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</tbody>
</table>
Results and Discussion

- Position **65 cM** in homology group 2
- Genetic variance associated with apomixis resulted in **81.6%**
- The mapping to **only one homology group** is probably associated to the region of apomixis (ASGR)

Ebina et al. (2005)  Vigna et al. (2016)
Conclusion and Perspectives

- A linkage map of higher resolution and more informative for *M. maximus*
- Mapping of the apomixis region
- Future projects mapping QTL
- Evolutionary studies on the genomic organization of this species and other forage grasses.
Collaborations and Acknowledgments

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Thank you for your attention!!!!