IFTBC
International Forage & Turf Breeding Conference
A Global Vision for Innovation

# 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

Embrapa Gado de Corte

UNICAMP

# Introduction

## 412 million acres

Fonte: pastagem.org

**Figure.** Pastures in Brazil (yellow).

# Introduction

❑ **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**

# Introduction

- **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**

***Traits***

**Evolution genome**

**Structure genome**

# Introduction

## Apomixis

- Asexual reproduction by seeds;

Progeny Tests

Embryo sac analysis

**Distinguish sexual and apomictic plants**

## Apospory-Specific Genomic Region (ASGR)

Dominant character → A single or few genes

**Markers intrinsically linked to ASGR**

# Introduction

- **SNP Markers and Linkage Mapping**
  - Single dose for one parent or both parents

**(Aaaa x aaaa)**

**(Aaaa x 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 tetraploidizad** ✖ **Apomictic genotype 'cv. BRS Mombaça'**

**136 F1 hybrids**

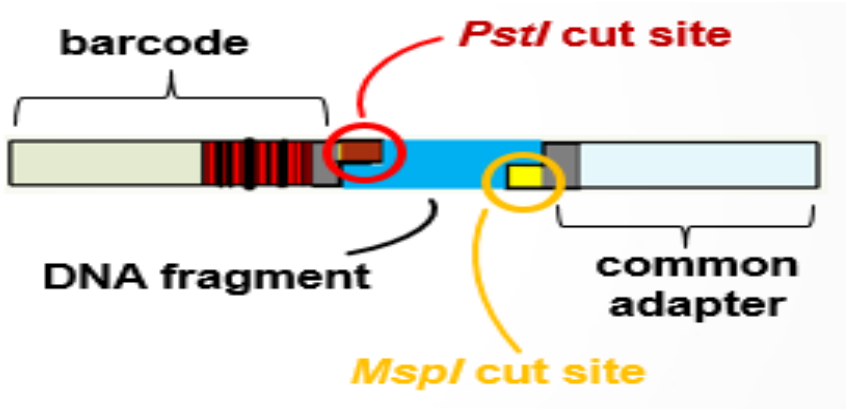
Fonte: Toledo-Silva, 2013.

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

# Methods

Extraction

GBS library




NGS Sequencing
NextSeq 500

Single-end reads
150 bp reads
400 million reads

# Methods

## Data analysis

### Sequencing quality

- *NGS QC Toolkit*

### SNP calling

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

### Allele dosage

- Deph ≥ 60 reads
- Dosage (*Updog)*

### Filter quality

- Monomorphic markers
- Missing data 25%

### Linkage analysis

- *TetraploidSNPMap*

# Methods

# Apomixis locus region

**Linkage map** ✚ **Inferring Phases** ✚ **Cytoembryological analysis**

❖ *TetraploidSNPMap*

〓 **Apomixis locus region**

❖ *TetraploidSNPMap QTL module*

# Results and Discussion

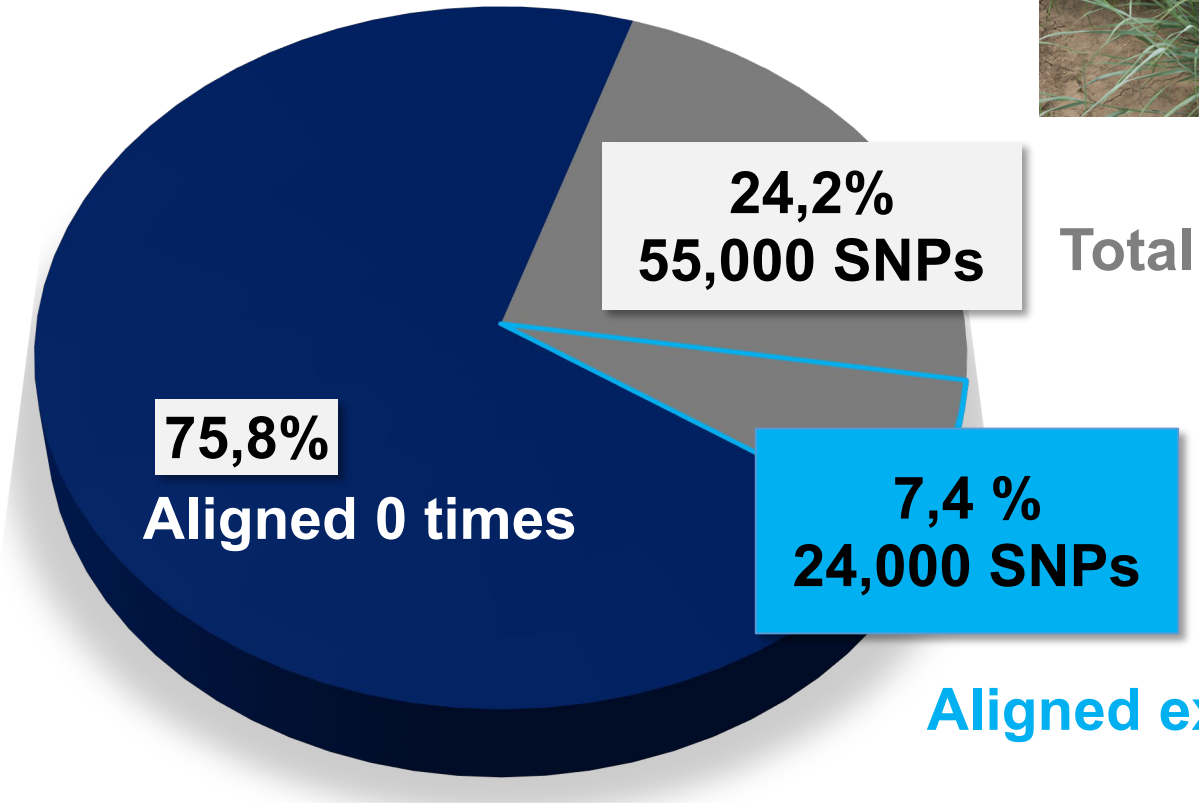
**Alignment**

**SNP calling**

**720 million reads of 138 individuals**

❖ Phred score 30

## Alignment to Switchgrass genome



# Results and Discussion

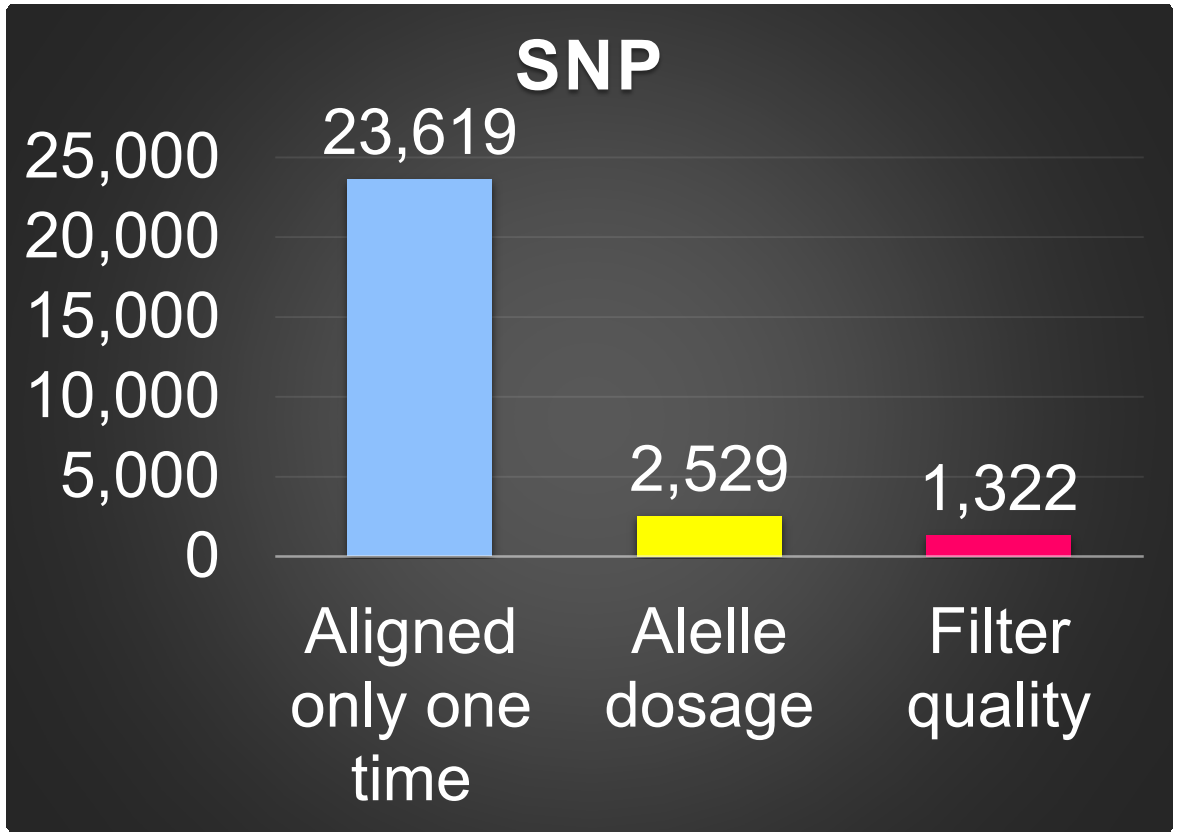
**Allele depth**

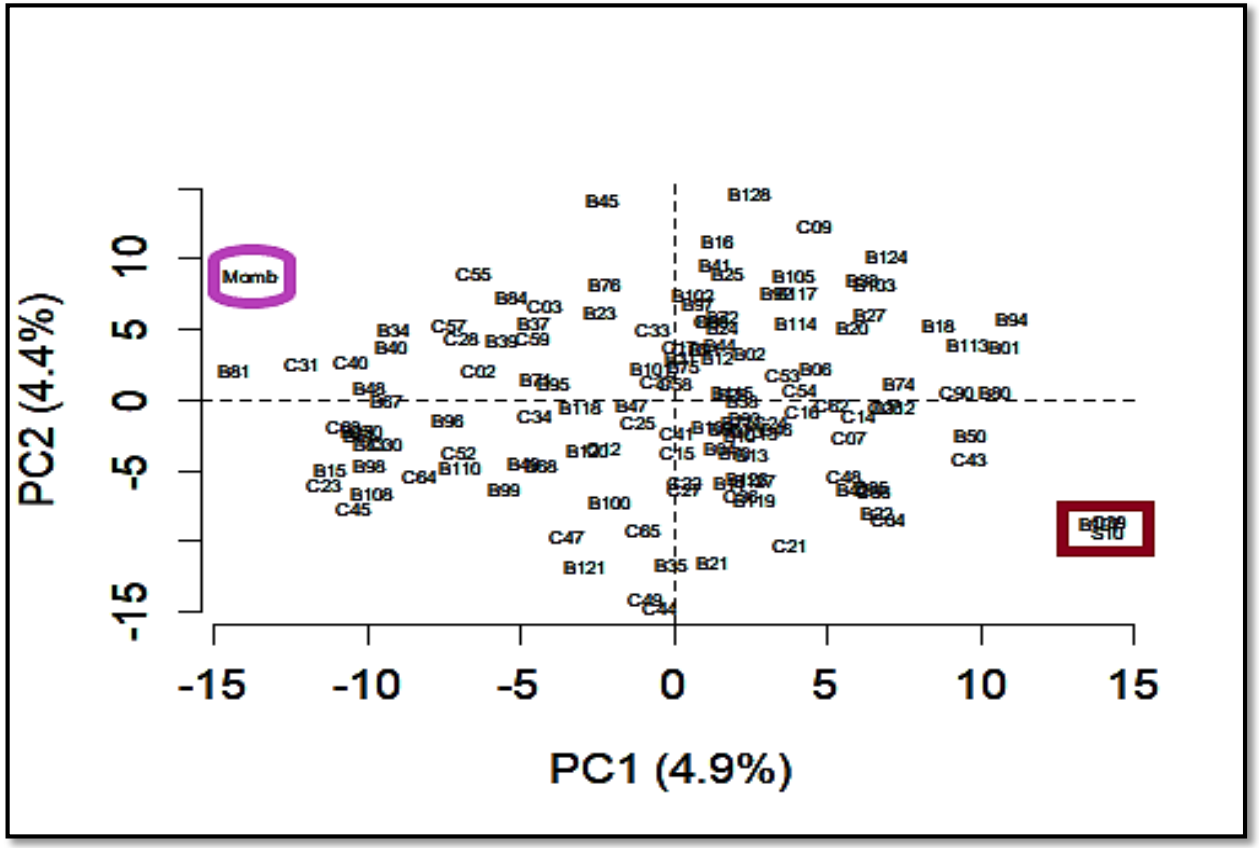
**Allele dosage**

Depth ≥ 60 reads

**Manual filtering**

Missing data ≥ 25%



# Results and Discussion

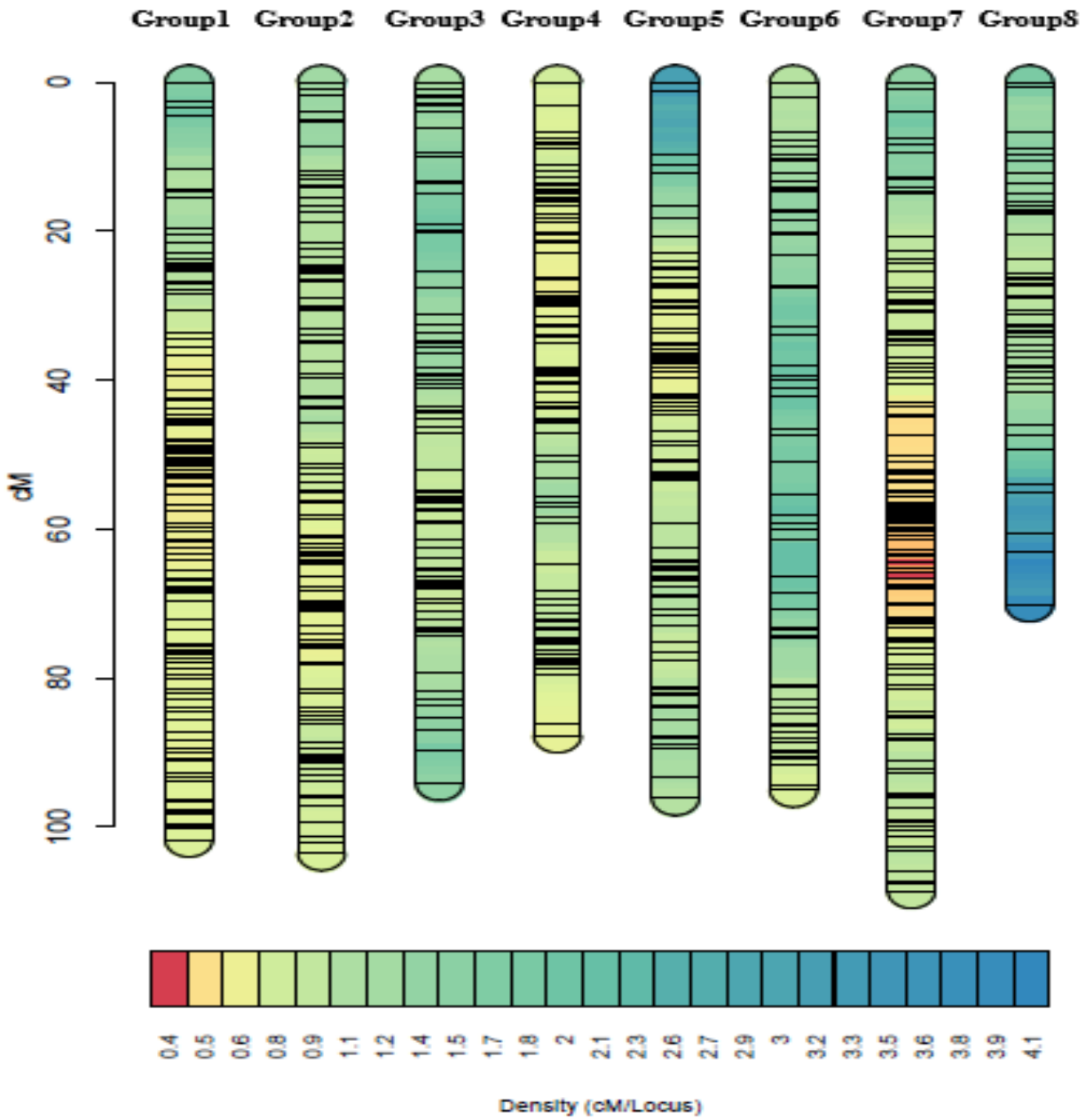
**858 SNP markers with multiple dose**

**Size of 756.7 cM**

**Average density 1.13 cM**

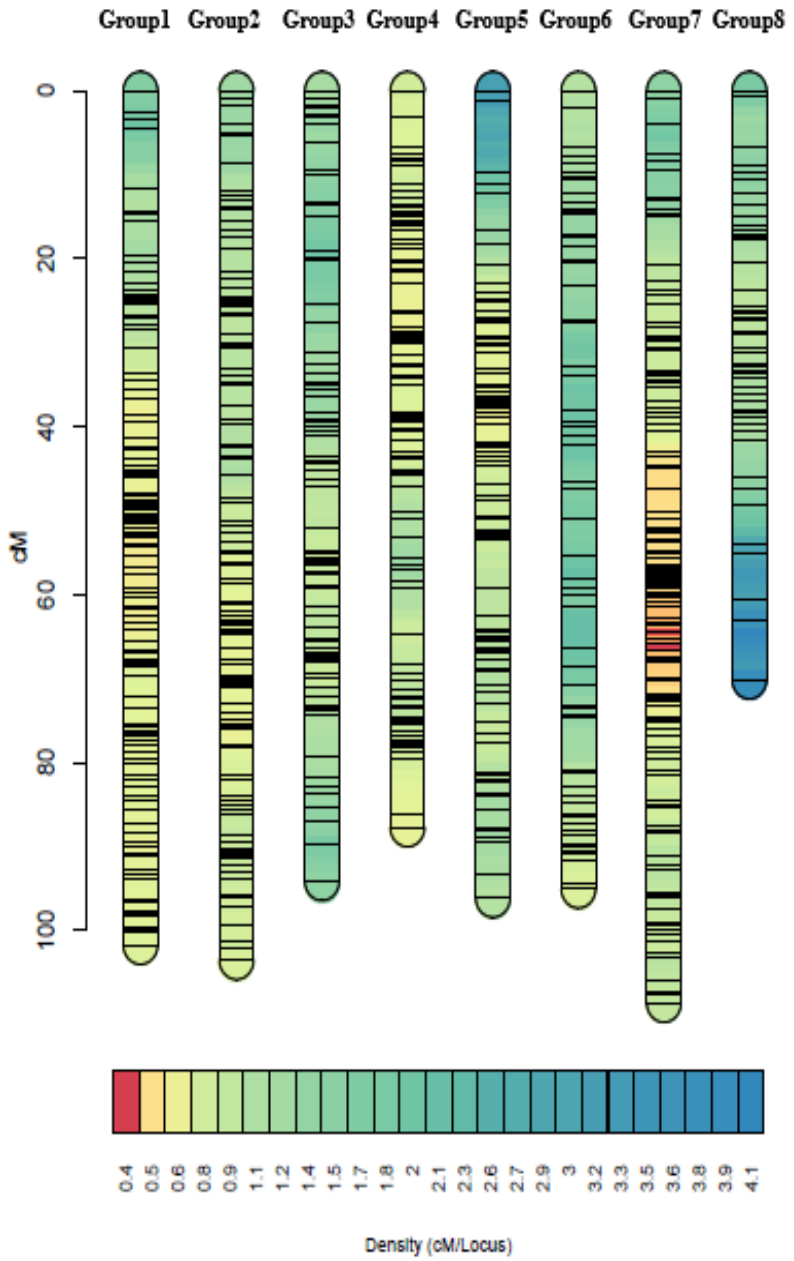
**Greater distance – 8.6 cM - Group 5**

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



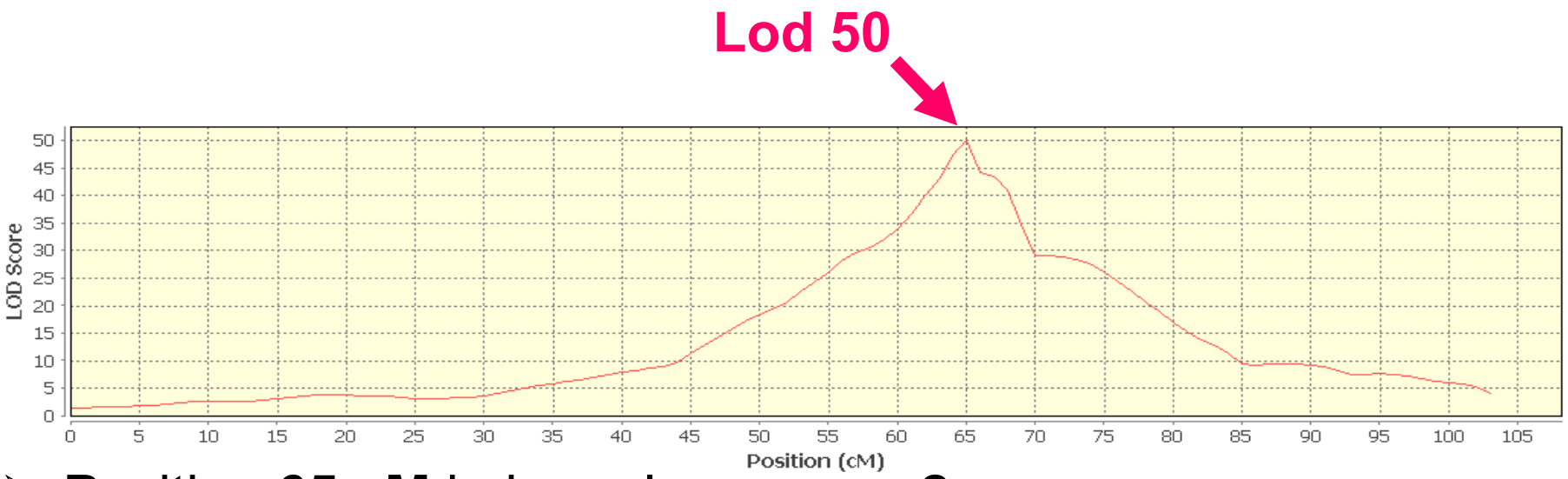
# Results and Discussion

| HG | SNP | Size (cM) | Longest distance between markers (cM) |
|---|---|---|---|
| **1** | 144 | 101.7 | 7.2 |
| **2** | 128 | 103.5 | 3.5 |
| **3** | 85 | 94.1 | 5.3 |
| **4** | 115 | 87.7 | 6.4 |
| **5** | 104 | 96.1 | 8.6 |
| **6** | 74 | 95.0 | 5.4 |
| **7** | **159** | **108.6** | **5.6** |
| **8** | **49** | **70.1** | **7.0** |



# Results and Discussion

## Apomixis




- 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.

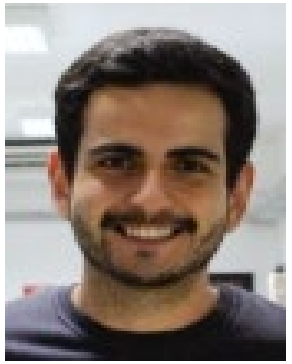
# Collaborations and Acknowledgments

Anete Souza

Rebecca Ferreira

Aline Moraes

Fernanda Oliveira



Felipe Martins

Letícia Lara

Antônio Garcia

Liana Jank

Mateus Santos

# Thank you for your attention!!!!

Embrapa FAPESP CAPES UNICAMP