Genome-wide polymorphisms in polyploids

*Paspalum* species of Plicatula group

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- Created in 1962
- Public university in the state of São Paulo
- 15% of Brazilian research

Forage grasses team
Prof. Anete Pereira de Souza - Coordinator

Brazilian Agricultural Research Corporation
Pasturelands in Brazil

- 170 M ha pastures
- 209 M cattle heads

“90% of diet”

- Brazil: one of the main beef and tropical forage seeds exporter in the world

Figure. Pasturelands areas in Brazil.
Pasturelands in Brazil

- Pastures: 172 mi
- Agriculture: 77 mi
- Forests: 100 mi
- Native: 52 mi
- Cultivated: 120 mi
Genus *Paspalum*

- **Native of South America**
- **High inter- and intra-specific variability in Brazil**
- **400 species, 214 in Brazil**
- **25 informal groups**
- **Germplasm**: Embrapa Pecuária Sudeste, São Paulo, Brazil.
Plicatula Group

- 30 species
- 57% of Embrapa’s GB
- Lack of correct identification hinders the use of germplasm
- Forage attributes (ex. *P. plicatulum*, *P. atratum*, *P. guenoarum*)
Genetic variation in polyploid forage grass: Assessing the molecular genetic variability in the *Paspalum* genus

Cidade et al, 2013

**SSR markers**
- *P. notatum* (Notata)
- *P. atratum* (Plicatula)

Exclusive bands among varieties of *P. notatum*

**Plicatula Group** - there was no separation
First microsatellite markers for *Paspalum plicatum* (Poaceae) characterization and cross-amplification in different *Paspalum* species of the Plicatula group

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- **P. atratum**
  1. Efficiency of loci amplification
  2. High number of exclusive bands

Genetic proximity of the species – sharing of alleles
Challenges in polyploid genotyping

- Example: Autotetraploid

- Allelic dosage classes:

  - homozygous
  - heterozygous
  - homozygous

- Gel Visualization

Bourke et al., 2018
High-throughput genotyping methods

- Restriction site-associated DNA sequencing (RAD-seq)
- Large number of SNPs
- Statistical methods for SNP genotype calling on polyploid species are critical
- Allele dosage – evolution for polyploid studies
- Large-scale phylogenies are possible
The aim of the study

To use a RAD-seq approach for the development of molecular markers to contribute with the classification of related species of Plicatula group.

- Developing and sequencing of RAD-seq libraries
- SNP calling
- Allele dosage estimation
- Phylogenetic Tree construction
RAD-seq Library Construction and Sequencing

DNA Extraction

Apomictic tetraploids

60 accessions
13 species

P. plicatulum

Enzyme SbfI
Size shear 600–700 bp

12 Different species

Enzyme SbfI
Size shear 400 bp

Sequencing

300 pb PE
MiSeq v3

150 pb PE
NextSeq 500
Genotyping and Data analysis

Alignment

1. Panicum virgatum
2. Panicum hallii
3. Setaria viridis
4. Setaria itálica
5. Urochloa ruziziensis (draft genome)
6. Urochloa humidicola (transcriptome)

Grasses

SNP Calling and Allele Dosage

Freebayes Software

Clustering analysis

R package adegenet - DAPC

Functional analysis

REVIGO - Gene Ontology terms
Results – Sequencing and SNP Calling

- 1st and 2nd sequencing: 78 million reads → 67 million reads filtered

- P. notatum Transcriptome: ~6.5 M reads with single alignment
  - 395,259 SNPs
  - 5,463 SNPs markers
    - ✓ 95% presence among the samples
    - ✓ depth greater than 480 reads (average of 8 reads per individual)
Results – SNP allele dosage

![Graph showing SNP Configuration with number of 2nd allele]
Results - Clustering analysis

Unrooted neighbor-joining tree based on PCA matrix data
Results - Functional analysis

**Biological Processes** related to the SNP markers regions

- **Development**
  - recognition of pollen
  - embryo sac egg cell differentiation
  - Carpel development

- **Defense response**
  - response to salt stress
  - response to red light

- **Cell wall**
  - Plant-cell wall organization

- **Regulation of proteins**
  - protein ubiquitination
  - Protein phosphorylation
  - Carbohydrate phosphorylation
Conclusions

- **First RAD-seq approach** for species of *Paspalum*

- Putative **functional SNPs markers** with allele dosage information

- Opened **new molecular perspectives** for these orphan species

**Ongoing research...**

- Alignment with grass genomes

- Phylogenetic Tree construction - search for more markers
Institutions involved

Dr. Bianca Vigna  Dr. Alessandra Fávero  Prof. Paul Hohenlohe  Prof. Gabriel Margarido

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