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

Paspalum atratum and *P. malacophyllum* pollen cryopreservation

Alessandra P. Fávero, Bianca B. Z. Vigna and Naiana B. Dinato

Lake Buena Vista, March 26th 2019

Paspalum

American Poaceae genus

400 species in the American continent
214 species in Brazil

High genetic diversity

Mainly tetraploid and apomictic

Agamic complexes

Informal botanical groups (Chase 1929)

R. D. Marra

L.A.R. Batista

Paspalum germplasm bank at Embrapa

430 accessions
- ~49 species;
-Registered cultivars:
-Forage: 2
-Turfgrass: 5

Paspalum forage breeding

> 50% from Plicatula Group (246 accessions)

2x sexual and 4x apomictic

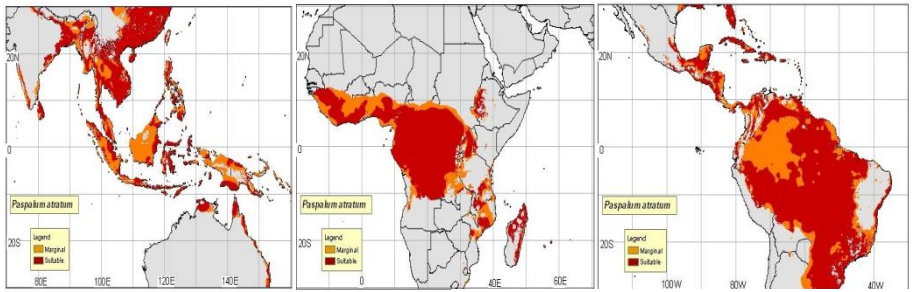
Paspalum atratum (atra paspalum)

Native to Brazil
Group Plicatula

Adapted to acidic soils
Tolerant to flooding
High seed production

Flowering in Mar-Apr
4x apomictic

Flora do Brasil 2020 -
http://floradobrasil.jbrj.gov.br



http://www.tropicalforages.info

Paspalum malacophyllum (ribbed paspalum)

Native to Brazil

Group Malacophylla

High palatability

High leaf area

Flowering in Fev-Mar

4x apomictic

Flora do Brasil 2020 -
http://floradobrasil.jbrj.gov.br

Paspalum forage breeding

Paspalum atratum x *Paspalum malacophyllum*

X

both late blooming but apomictic

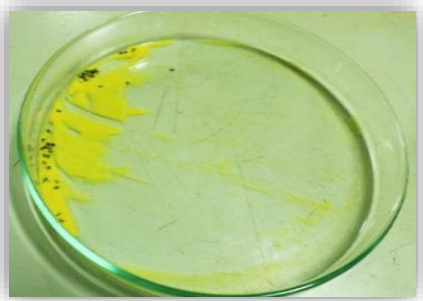
For interspecific hybridization in the genus
- sexual tetraploid must be available
- parental flowering time must be synchronized

manipulating photoperiod and temperature

Pollen conservation

Feasible approach

- to preserve pollen until female parent flowering
- to conserve genetic diversity



Research addressing pollen storage began in the late 19th century

Horticultural, ornamental and forest crops

Few studies with grasses

Pollen cryopreservation

Cryopreservation is the conservation of biological material in liquid nitrogen at - 196 ºC or in its vapor phase at - 150 ºC

Cell water content is critical to succeed

At 0ºC ice crystals are formed, damaging tissues

When high, pollen dehydration is necessary

↳ salts and silica gel

Success → Time

Humidity water content between 7% and 20%

Temperature between - 5 ° C to - 196 ° C

Objectives

To determine *P. atratum* and *P. malacophyllum* pollen stainability after 12-month storage in liquid nitrogen, in order to allow the crossing of these species with asynchronous flowering species





Material

Paspalum atratum

BGP 98

cv. Pojuca/Suerte

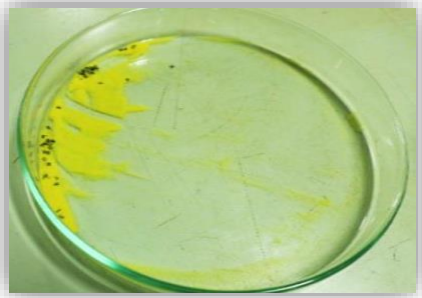
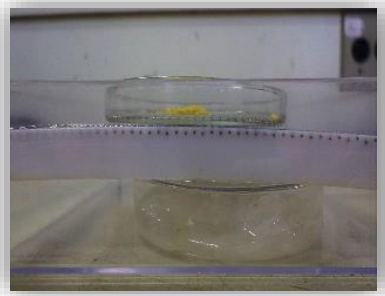
BGP 308

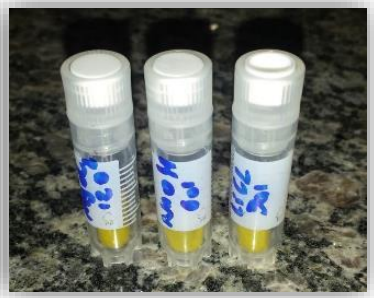
Paspalum malacophyllum

BGP 6

BGP 293

Methods




2,3,5-triphenyl tetrazolium chloride (TTC) 0,25%

Methods

Dehydration agents

RESEARCH

Storage of Bahiagrass Pollen at Different Temperatures

Naiana Barbosa Dinato, Izulmé Rita Imaculada Santos, Eduardo Leonardecz, Byron L. Burson, Camilo L. Quarín, Ailton Ferreira de Paula, and Alessandra Pereira Fávero*

Published in Crop Sci. 58:2391–2398 (2018).
doi: 10.2135/cropsci2018.03.0164

Treatments:

- Fresh
- Cryopreserved
  - Non dehydrated
  - Dehydrated
    - Silica gel
      - 30 minutes
      - 60 minutes
      - 120 minutes
    - LiCl
      - 30 minutes
      - 60 minutes
      - 120 minutes

Statistical analysis

3 replicates - mean
% stained pollen grains
Boxplot analysis
Scott-Knox test 5%

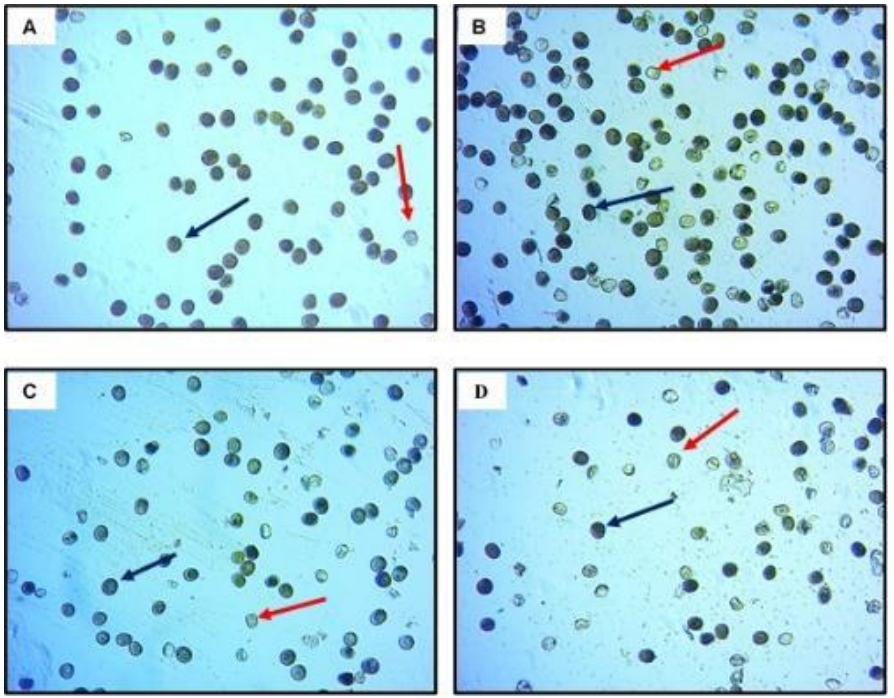
Period of storage 1, 10, 30, 90, 180, 270, 365 days

Pollen Stainability

Pollen stained with TTC 0,25% after cryopreservation for 24h

A) Control (Fresh pollen)
B) LiCl at 30 minutes
C) Silica gel at 120 minutes
D) Non dehydrated pollen

Blue arrow: stained pollen - viable
Red arrow: non stained pollen - non-viable



Conclusions

LiCl for 30 minutes and silica gel for 120 minutes are the best dehydrating agents and treatment times

After treatments, pollen stainability was better than the non-hydrated treatment and above 50%

No difference among genotypes of *Paspalum*

No influence of time of storage on the pollen stainability





Ongoing research

BGP 393 (*P. urvillei*)

x

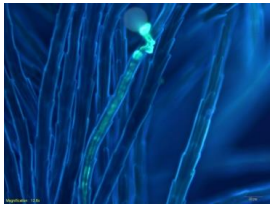
P. malacophyllum

(*P. plicatulum* 4PT x *P. guenoarum* cv. Azulão)^{4x}

x

P. atratum

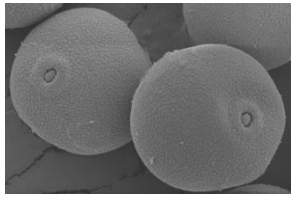
Ongoing research



In vivo germination by fluorescence microscopy

Identification of hybrids using molecular markers

Pollen morphology



Applications

Urochloa spp.

Guineagrass (*Megathyrsus maximus* syn. *Panicum maximum*)

St. Augustine grass (*Stenotaphrum secundatum*)

Financial Support

CAPES

Embrapa

UNIPASTO
ASSOCIAÇÃO PARA O FOMENTO À PESQUISA DE MELHORAMENTO DE FORRAGEIRAS

Collaborators

Alessandra Fávero

Naiana Dinato

UNIVERSIDAD NACIONAL DEL NORDESTE

Camilo Quarin
Carlos Acuña

Thank you!

bianca.vigna@embrapa.br