ADDENDUM TO
THE USE OF MOLECULAR MARKERS (SNP) FOR MAIZE DUS TESTING IN FRANCE (2013 TO 2016)

prepared by an expert from France

Disclaimer: this document does not represent UPOV policies or guidance

The Annex to this document contains a copy of a presentation on “The use of SNP molecular markers for maize DUS testing in France from 2013 to 2016” to be made by an expert from France at the sixteenth session of the Working Group on Biochemical and Molecular Techniques, and DNA-Proﬁling in Particular (BMT).

[The Annex follows]
The use of SNP molecular markers for maize DUS testing in France from 2013 to 2016

Experts from France
BMT 2017

Context

- The Use of Molecular Markers at BioGEVES for maize DUS testing
  - ≈300 SNP markers
  - Renewal of reference material
  - Checking hybrid conformity
  - Management of the reference collection (UPOV model 2)
**Context**

- The Use of Molecular Markers at BioGEVES for \textit{maize} DUS testing
  - \( \approx 300 \) SNP markers
  - Renewal of reference material
  - Checking hybrid conformity
  - Management of the reference collection (UPOV model 2)

**Management of the Reference Collection (UPOV model 2)**

- Each candidate variety must be compared with all other varieties under study as well as with all the varieties belonging to the reference collection

\[
\text{Total number of pairs} = n_{\text{candidate}} \times (n_{\text{candidate}} + r_{\text{collection}}) - n_{\text{candidate}}
\]

- Very large number of pairs (about 1 Million pairs/year)

- Methods and tools necessary to reduce the number side by side comparisons in the field
  - Morphological description of the DUS characteristics
  - Isoenzyme electrophoresis (until 2012)
  - SNP Genotyping (since 2013)
Reminder: Methods and Tools Used to Reduce Field Implantation

DATA
> Evaluation of Morphological traits

> DNA genotyping (SNP)

GAIA software
Developed by OSV5

> Morphological distance

R software

> Genetic distance

What to sown?
(SPV Mode 2)

Reminder: Zones And Thresholds (Model 2)

2 steps: Combination morphological weight/genetic distance

1: Morphological distance (GAIA)
   - GAIA < 0.5: Super distinct pairs
   - GAIA > 0.5: To put in the field

2: Genetic distance (GD)
   - GD < 0.2: To put in the field
   - GD > 0.2: Distinct varieties

No molecular analysis in the area of GAIA < 2
Maize DUS Time Line

Candidate Varieties

Year 1
- Description of DUS characteristics + Genotyping
- GAIA analysis
- Genetic Distance Calculation
  - Super distinct
  - Distinct

Withdrawn/Refused

Year 2
- Side by side comp. trials
  - Distinct

Year 3
- Side by side comp. trials
  - Distinct

REFUSED

Goals of this study

- Collect and consolidate available data since the use of the SNPs for Maize DUS testing (2013 to 2016)
- Highlight the efficiency of the model currently used at BioGEVES to reduce the number of pairs implanted in the field
- Which evolution of this model?
Our 4-years experience on model 2 approach in maize (2013 to 2016 data)

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280 new candidate inbred lines
(>181 candidates still under study)

3657 varieties belonging to the reference collection of GEVES

= 1 152 759 side by side comparisons
- 99% GAIA savings

7004 pairs (GAIA < 6)
- 94% molecular savings

429 to grow side by side
Report of the DUS Data (2013 to 2016)

2014

363 new candidate inbred lines
(>262 candidates still under study)

3702 varieties belonging to the reference collection of GEVES

= 1,550,339 side by side comparisons

6365 pairs (GAIA<6)
- 91% molecular savings

559 to grow side by side

2015

223 new candidate inbred lines
(>273 candidates still under study)

3726 varieties belonging to the reference collection of GEVES

= 918,983 pairs to compare side by side

7448 pairs (GAIA<6)
- 93% molecular savings

504 to grow side by side
Report of the DUS Data (2013 to 2016)

208 new candidate inbred lines
(148 candidates still under study)

3814 varieties belonging to the reference collection of GEVES

= 867 152 pairs to compare side by side

5020 pairs (GAIA < 6)
- 91% molecular savings

448 to grow side by side

2013 to 2016 data compilation

- 21 pairs required one more year of study
- Located in the area GAIA < 6 and GD ≤ 0.2
- None have been identified in the zone GAIA < 2 and GD > 0.2
- New threshold to reduce the number of field implantations?
Savings in Terms of Pairs Implanted in the Field?

Simulations with different thresholds

<table>
<thead>
<tr>
<th>Threshold</th>
<th>2013</th>
<th>2014</th>
<th>2015</th>
<th>2016</th>
<th>Average</th>
<th>Savings</th>
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<tbody>
<tr>
<td>0.6</td>
<td>420</td>
<td>446</td>
<td>499</td>
<td>444</td>
<td>448</td>
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<tr>
<td>0.2</td>
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<td>227</td>
<td>227</td>
<td>227</td>
<td>227</td>
<td>28%</td>
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</tbody>
</table>
Conclusions

- We have confirmed the efficiency of Model 2 to decrease the number of side by side comparisons.
- Data collected since 2013 (4 years) do not show any close pair in the zone GAIA < 2 and for GD > 0.2.
- Which new threshold could be set up with good compromise between safety and field savings?

<table>
<thead>
<tr>
<th>Thresholds</th>
<th>0.2</th>
<th>0.3</th>
<th>0.4</th>
<th>0.5</th>
<th>0.6</th>
</tr>
</thead>
<tbody>
<tr>
<td>Field Savings</td>
<td>28%</td>
<td>22%</td>
<td>15%</td>
<td>8%</td>
<td>1%</td>
</tr>
</tbody>
</table>

Thank you