WORKING GROUP ON BIOCHEMICAL AND MOLECULAR TECHNIQUES AND DNA PROFILING IN PARTICULAR

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ADDENDUM

STANDARDS FOR HELPING TO DETERMINE EDV STATUS IN MAIZE (ZEA MAYS L.) USING SSR’S AND FUTURE PROSPECTS USING SNP’S

Document prepared by experts from the United States of America
Standards for helping to determine EDV status in maize using SSRs and future prospects using SNPs

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American Seed Trade Association
Corn Variety Identification Sub-Committee

Selection and evaluation of panels of SSR loci

ASTA (American Seed Trade Association) have selected a set of 285 SSR loci for EDV

- Selection based on:
  - Genetic conformity
  - Expected Heterozygosity (informative)
  - Genome coverage
  - Scoring ease using an inexpensive assay

- 150 sub-set (Core Set 1) for efficiency, additional 135 use as needed.

- Published in Crop Science


- 285 marker set posted on ASTA web site (www.amseed.org)

UFS (French Maize Breeders, formerly SEPROMA)

selected a set of 223 SSR with a core set of 163 for EDV
Evaluation of SNPs for genetic similarity in maize

Maize breeders are moving to SNPs

- Throughput
- Cost efficiencies

Necessitates the need to evaluate SNP utility in EDV

Objective

- Evaluate SNP’s for potential use in EDV
  - Considered a “primary step” before EDV thresholds could be determined
  - Genetic associations with pedigree relatedness and SSRs
  - Number of SNPs necessary

Inbreds Used

- 98 inbreds selected by ASTA members
  - Public sources
    - Public material
    - Off-PVP
  - Same material used in SSR panel evaluation
- 30 inbred sub-set profiled with SEPRONAs
- 163 core set
  - Reduced inbred set due to cost
  - Maintained diversity of 98 inbred set.
Marker Sets
-SSR Sets and SNP Assay

SSRs
- 150 ASTA public set
- 163 SEPROMA SSRs

SNPs
- Started with 768 public set
  - Profiled by Pioneer
    - Aliquots of same DNA used for published SSR panel
    - Illumina GoldenGate® assay
  - Data subject to quality control steps
    - Thresholds for both inbreds and markers
      - 10% hets
      - 70% data coverage
    - 80 of the 98 inbreds used in analysis
    - 26 of 30 SEPROMA sub-set

Marker Sets
-SNP Set Selections

6 Subsets evaluated
- 601 Set
  - Passed quality control
- 447 Set
  - Removed unmapped and expected heterozygosity = 0
- 306 Set
  - Removed loci mapped to same location, retaining highest expected heterozygosity
- 204 Set
  - Removed loci mapped within 10 cM of another, while maintaining genome coverage & previous expected heterozygosity average
- Maintain genome coverage (with increasing intervals between loci) & previous expected heterozygosity average
- 83 Set
- 42 Set
### Marker Sets - Expected Het. & Genome Coverage

<table>
<thead>
<tr>
<th>Marker Set</th>
<th>Average (range) expected heterozygosity for 80 inbred set</th>
<th>Average (range) expected heterozygosity for 26 inbred subset</th>
<th>Genome coverage (%)</th>
<th>Average (range) marker distances (cM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>150 ASTA SSR</td>
<td>0.53 (0.03 – 0.79)</td>
<td>0.49 (0.00 – 0.80)</td>
<td>89.0</td>
<td>47.2 (0-196.0)</td>
</tr>
<tr>
<td>163 SEPROMA SSR</td>
<td>NA</td>
<td>0.65 (0.00 – 0.88)</td>
<td>88.2</td>
<td>44.1 (0-206.1)</td>
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<tr>
<td>83 SNP</td>
<td>0.35 (0.00-0.50)</td>
<td>0.34 (0.00-0.50)</td>
<td>88.4</td>
<td>15.1 (0-112.8)</td>
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<tr>
<td>204 SNP</td>
<td>0.41 (0.03-0.50)</td>
<td>0.39 (0.00-0.50)</td>
<td>88.0</td>
<td>23.3 (0-117.8)</td>
</tr>
<tr>
<td>306 SNP</td>
<td>0.41 (0.03-0.50)</td>
<td>0.40 (0.00-0.50)</td>
<td>87.2</td>
<td>33.9 (6.4-117.8)</td>
</tr>
<tr>
<td>63 SNP</td>
<td>0.48 (0.30-0.5)</td>
<td>0.46 (0.15-0.50)</td>
<td>83.5</td>
<td>89.5 (13.8-240.3)</td>
</tr>
<tr>
<td>22 SNP</td>
<td>0.48 (0.34-0.5)</td>
<td>0.47 (0.26-0.50)</td>
<td>74.8</td>
<td>186.7 (44.6-408.8)</td>
</tr>
</tbody>
</table>
Van Inghelandt et al., (2010) used the CV of genetic distances to compare marker type and number.

- Independence from polymorphism and marker number factors between individual comparisons
- Bootstrap analysis (1000 reps) of Rogers Distance in NTSYSpc version 2.21
- Created randomly chosen SNP sets, same in number, to compare against sets selected for informativeness and genome coverage.
CV Comparison (Roger’s D)

- CV of Genetic Distances
- Randomly Selected SNPs
- Expected Het & Coverage SNPs

Pairwise Correlations

- Roger’s distance based
- Pair distances for different marker sets and pedigree relatedness were correlated ($R^2$)
- Pedigree Relatedness
  - Numerous pairs are not known to be related
  - Inbred pairs <0.25 removed to avoid spurious results
### Pairwise Correlations (R²)

#### Pedigree Relatedness

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<tr>
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<th>Pedigree Relatedness</th>
<th>150 ASTA SSR</th>
<th>163 SEPROMA SSR</th>
<th>601 SNP</th>
<th>447 SNP</th>
<th>306 SNP</th>
<th>204 SNP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pedigree Relatedness</td>
<td>0.38 NA 0.44 0.46 0.46 0.46</td>
<td>0.44 NA 0.55 0.58 0.61 0.59</td>
<td>0.66 0.6 0.75 NA NA NA NA</td>
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<tr>
<td>150 ASTA SSR</td>
<td>0.42 NA 0.55 0.58 0.61</td>
<td>0.55 NA 0.61 0.64 0.66 0.68</td>
<td>0.49 0.56 0.75 0.98 0.98 0.92</td>
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<td>0.52 0.61 0.82 0.97 0.99</td>
<td>0.61 0.64 0.66 0.68 0.69 0.71</td>
<td>0.53 0.65 0.82 0.97 0.99 0.98</td>
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<tr>
<td>447 SNP</td>
<td>0.66 0.69 0.97 0.99 0.98</td>
<td>0.69 0.72 0.75 0.82 0.83 0.85</td>
<td>0.56 0.66 0.82 0.95 0.97 0.98</td>
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**80 inbreds**

**26 inbreds**

#### 150 ASTA SSR’s / 306 SNP’s

**EDV Zone**

**82% Similarity**

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**BMT/12/14 Add.**

**page 8**
**Summary**

- SNPs will be useful in EDV determination
  - Demonstrate high discriminating power
  - Accurately reflect inbred associations with known pedigrees
- SNP sets selected for informativeness and genome coverage had lower CVs than randomly chosen sets of the same number of SNPs per set
- 204 and 306 SNP sets
  - Gave equivalent CVs to the 150 ASTA and 163 SEPROMA SSR sets
  - Had the best correlations to pedigree relatedness and SSR distances
Future Experiments

SNP chip development
- Illumina has developed 60,000 public SNP chip
- Leverage for SNPs in EDV with
  - Established EDV SSR sets
  - Inbred sets
  - Additional closely related material in EDV similarity zones

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