UPOV

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# ADDENDUM TO CORN HYBRID PARENTAL IDENTIFICATION: THE USE OF HYBRID MONOMORPHIC PROFILE COMPARED TO PERICARP GENOTYPING

*Document prepared by an expert from the Seed Association of the Americas (SAA)*

*Disclaimer: this document does not represent UPOV policies or guidance*

The Annex to this document contains a copy of a presentation on “Corn Hybrid parental identification: The use of Hybrid Monomorphic Profile compared to Pericarp Genotyping”, prepared by an expert from the Seed Association of the Americas (SAA), which was made at the seventeenth session of the Working Group on Biochemical and Molecular Techniques and DNA-Profiling in Particular (BMT).

[Annex follows]

ANNEX

CORN HYBRID PARENTAL IDENTIFICATION: THE USE OF HYBRID MONOMORPHIC PROFILE COMPARED TO PERICARP GENOTYPING

Presentation prepared by an expert from the Seed Association of the Americas (SAA)

# Corn Hybrid parental identification:
# **The Use of Hybrid Monomorphic Profile Compared to Pericarp Genotyping**

**BMT- Sep 12th , 2018 – Montevideo UY**

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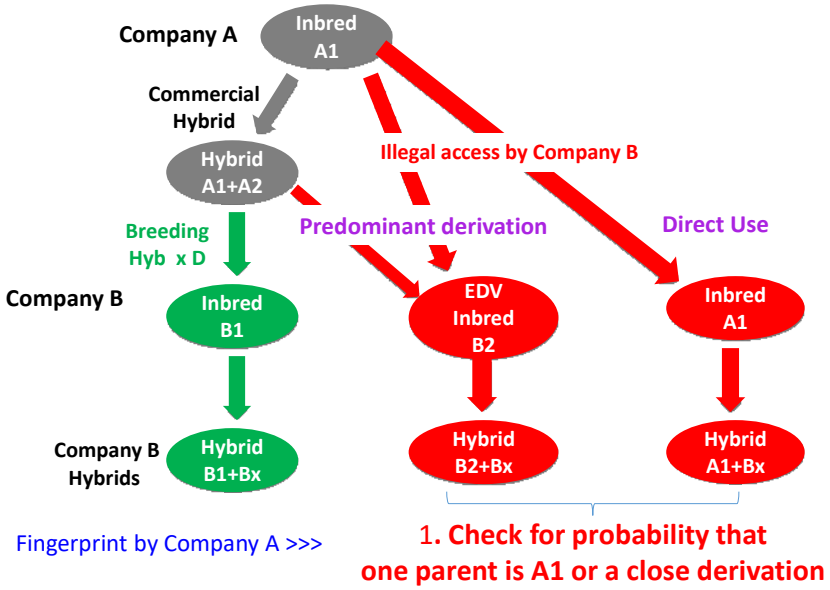
Seed Association of Americas Molecular Marker WG - Chair
SAA IP WG - Vice Chair

## Types of Intellectual Property Issues with Hybrid Crops

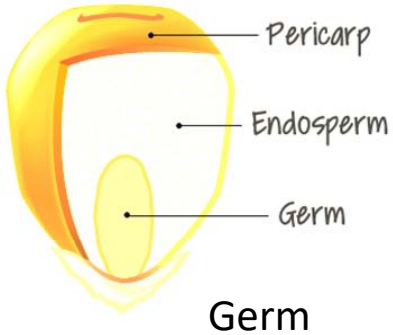


## Kernel Components Genetic Contribution:

Different tissues offer Opportunities to Detect Parental Genotypes

1) Genotyping Hybrid Endosperm

- **Maternal (2N)**
- **Paternal (1N)**




Germ

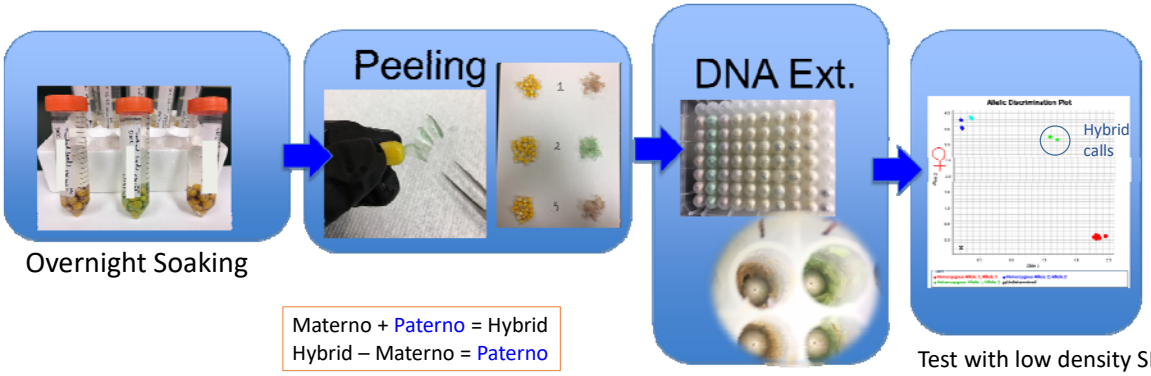
- **Maternal (1N)**
- **Paternal (1N)**

2) Genotyping the Hybrid Pericarp

- **Maternal (2N)**

## Pericarp Peeling, DNA Extraction, Pre-SNP Testing, Genotyping

Lorie Nguyen




Overnight Soaking

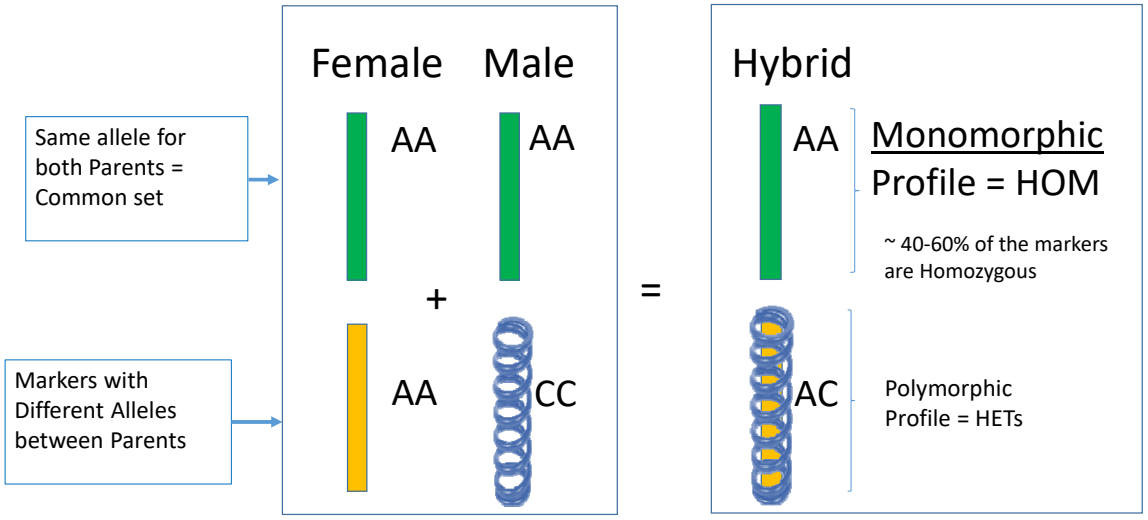
Materno + Paterno = Hybrid
Hybrid – Materno = Paterno

Test with low density SNP platform before GBS genotyping to check for contamination

**GENETIKA, vol 28, No. 3, 137-150, 1996.**
D. Stojsin et al.: Genetic Relationship among Corn Hybrids and parents based on RAPD analyses of pericarp and embryo DNA

## What is the Hybrid Monomorphic Profile?

List of markers with the same alleles for both male and female parent




## Monomorphic Profile- How to use it?

Competitor Hybrid Monomorphic Profile is tested against proprietary inbreds

**Competitor Hybrid Fingerprint** | **Proprietary Inbreds – FP Data**

Marker set

| | M1 | M2 | M3 | M4 | M5 | M6 | M7 | M8 | M9 | M10 |
|---|---|---|---|---|---|---|---|---|---|---|
| Hybrid X | AA | AT | GG | CG | AT | CG | CC | AA | CC | CG |

| | M1 | M2 | M3 | M4 | M5 | M6 | M7 | M8 | M9 | M10 |
|---|---|---|---|---|---|---|---|---|---|---|
| Inbred A | AA | AA | GG | GG | TT | CC | CC | AA | CC | GG |
| Inbred B | TT | TT | CC | CC | AA | GG | CC | TT | CC | CC |
| Inbred C | AA | AA | CC | CC | AA | CC | GG | TT | GG | GG |
| Inbred D | AA | TT | CC | GG | TT | CC | GG | AA | GG | GG |
| Inbred E | TT | AA | GG | GG | TT | GG | GG | TT | CC | GG |

- Estimate probability of a particular inbred being present in the hybrid combination
- Test parentage hypothesis . By using thousands of SNP markers it is possible to establish parentage relationship with high probability (Example: Infinium 50 K Maize SNP chip)

Goal: Establish that MMP is sufficiently comparable to Pericarp

Experimental Design: Confirming Parental contribution to hybrid kernel

I. Materials:

- Proprietary Hybrids and their Parental Lines
- Genotyping with:
  - Fingerprinting - FP (Infinium Chip): Endosperm (40K)
  - Genotyping by Sequencing (GBS): Pericarp and Endosperm (~1K SNPs)

## Experimental Design

Parental contribution to hybrid kernel components

II. Quality Check of Genotyping Results:

Expectation

1. Pericarp highly Homozygous = female inbred
2. Endosperm highly Heterozygous – different heterotic group cross
3. Inbreds - highly Homozygous = inbreeding

III. Comparisons Amongst Hybrid Components:

1. Compare Pericarp to Male and Female Parents: GBS.
   Expectation → Highly Similar to Female only
2. Monomorphic Profile and Parental lines: GBS and Infinium.
   Expectation → Highly Similar to Both, but not to other materials
3. Compare MMP to Pericarp: GBS.
   Expectation → Highly Similar to Each Other

IV. Summary on Hybrid Monomorphic Profile Results

Expectation

## II. 1. Hybrid Pericarp highly homozygous

### QC: Pericarp GBS Results

"-P" = Pericarp

| Coded Sample Name | Parent 1 | Parent 2 | Line Type | Total datapoints | % Homozygous calls |
|---|---|---|---|---|---|
| DK100-P-1 | INB23 | INB24 | Hybrid | 1076 | 97% |
| DK200-P-10 (source 2) | INB21 | INB22 | Hybrid | 1248 | 54% |
| DK200-P-11 (source 2) | INB21 | INB22 | Hybrid | 1249 | 54% |
| DK200-P-3 (source 1) | INB21 | INB22 | Hybrid | 1029 | 75% |
| DK200-P-5 (source 2) | INB21 | INB22 | Hybrid | 812 | 98% |
| DK300-P-2 | INB25 | INB26 | Hybrid | 1060 | 88% |
| DK400-P-4 | INB21 | INB27 | Hybrid | 1052 | 90% |
| DK500-P-6 | INB28 | INB29 | Hybrid | 1130 | 97% |
| DK600-P-7 | INB30 | INB31 | Hybrid | 1198 | 97% |
| DK700-P-8 | INB28 | INB32 | Hybrid | 1206 | 98% |
| DK800-P-9 | INB28 | INB33 | Hybrid | 1074 | 94% |

Endosperm Contamination?


Endosperm

400X stained with iodine and yellow food coloring

Expectation

## II. 2. Hybrid Endosperm expected to be highly heterozygous
## II.3. Inbred Endosperm expect to be highly homozygous

### QC: Endosperm GBS Results

"-E" = Endosperm

| Coded Sample Name | Parent 1 | Parent 2 | Line Type | Total datapoints | % Homozygous calls |
|---|---|---|---|---|---|
| DK100-E-12 | INB23 | INB24 | Hybrid | 1245 | 62% |
| DK200-E-14 (source 1) | INB21 | INB22 | Hybrid | 1247 | 55% |
| DK200-E-16 (source 2) | INB21 | INB22 | Hybrid | 1256 | 55% |
| DK400-E-15 | INB21 | INB27 | Hybrid | 876 | 54% |
| DK500-E-17 | INB28 | INB29 | Hybrid | 1240 | 48% |
| DK600-E-18 | INB30 | INB31 | Hybrid | 1249 | 44% |
| DK700-E-19 | INB28 | INB32 | Hybrid | 1246 | 49% |
| DK800-E-20 | INB28 | INB33 | Hybrid | 1255 | 49% |
| INB21-E | - | - | Inbred | 1218 | 99% |
| INB22-E | - | - | Inbred | 1218 | 95% |
| INB23-E | - | - | Inbred | 1186 | 99% |
| INB24-E | - | - | Inbred | 1183 | 99% |

Expectation

## III. 1. Hyb Pericarp highly similar to Female Parent:

GBS Results

"-P" = Pericarp

| Hybrid: Pericarp sample (GBS Only) | INBRED: Endosperm Sample (GBS or FP) | Pericarp Homoz and Parent % Sim (All calls) | # Mismatch | % Pericarp & Parent Match (Hom Calls only) |
|---|---|---|---|---|
| DK100-P-1 | INB23-E-GBS | 887 | 6 | 99% |
| DK200-P-3 (source 1) | INB21-E-GBS | 743 | 18 | 98% |
| DK200-P-5 (source 2) | INB21-E-GBS | 765 | 10 | 99% |
| DK200-P-11 (source 2) | INB21-E-GBS | 629 | 8 | 99% |
| DK300-P-2 | INB25-E-FP | 936 | 21 | 98% |
| DK400-P-4 | INB21-E-GBS | 906 | 12 | 99% |
| DK500-P-6 | INB28-E-FP | 1082 | 20 | 98% |
| DK600-P-7 | INB30-E-FP | 1144 | 13 | 99% |
| DK700-P-8 | INB28-E-FP | 1171 | 14 | 99% |
| DK800-P-9 | INB28-E-FP | 998 | 17 | 98% |
| **AVERAGE Comparison Pericarp to FEMALE** | | 926 | 14 | 98% |

✓

Parent data used for comparison showing as "–FP" is from a Fingerprinting run

Expectation

## III. 1. Hyb Pericarp highly similar to Female only:

MALE INBRED GBS Results

"-P" = Pericarp

| Hybrid: Pericarp sample (GBS Only) | INBRED: Endosperm Sample (GBS or FP) | Pericarp Homoz and Parent % Sim (All calls) | # Mismatch | % Pericarp & Parent Match (Hom Calls only) |
|---|---|---|---|---|
| DK100-P-1 | INB24-E-GBS | 882 | 316 | 64% |
| DK200-P-3 (source 1) | INB22-E-GBS | 712 | 188 | 74% |
| DK200-P-5 (source 2) | INB22-E-GBS | 753 | 376 | 50% |
| DK200-P-11 (source 2) | INB22-E-GBS | 1191 | 609 | 49% |
| DK300-P-2 | INB26-E-FP | 934 | 462 | 51% |
| DK400-P-4 | INB27-E-FP | 848 | 381 | 55% |
| DK500-P-6 | INB29-E-FP | 1076 | 606 | 44% |
| DK600-P-7 | INB31-E-FP | 1142 | 706 | 38% |
| DK700-P-8 | INB32-E-FP | 1141 | 645 | 43% |
| DK800-P-9 | INB33-E-FP | 981 | 502 | 49% |
| **AVERAGE Comparison Pericarp to MALE** | | 966 | 479 | 52% |

??

✓

Parent data used for comparison with –FP data is from a Fingerprinting run

Expectation

## III. 2. MMP highly similar to both Female and Male

GBS Results

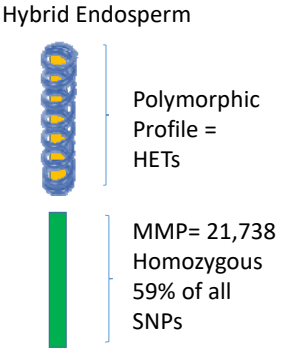
| Hybrid Pericarp Sample | Parent Comparison | INBRED Endosp MMP | Total Calls in Comparison | INB:Total Mismatch to Hybrid | INB and Hyb Monom. Profile % Sim |
|---|---|---|---|---|---|
| DK100-P-1 | Female | INB23-E | 695 | 6 | 99% |
| | Male | INB24-E | 694 | 9 | 99% |
| DK200-P-3 (source 1) | Female | INB21-E | 641 | 14 | 98% |
| | Male | INB22-E | 626 | 37 | 94% |
| DK200-P-5 (source 2) | Female | INB21-E | 638 | 0 | 100% |
| | Male | INB22-E | 627 | 41 | 93% |
| AVERAGE Comparison MMP to FEMALE | | | 658 | 7 | 99% |
| AVERAGE Comparison MMP to MALE | | | 649 | 29 | 95% |

Expectation

## II. 2. MMP highly similar to Female and Male Parents

(but not to other materials) – FP Results

| Match inbreds to MMP of DK200-E-Source 2 | Total Overlap with MMP | Mismatched | % Sim to MMP |
|---|---|---|---|
| FEMALE: INB21 | 21,091 | 302 | 99% |
| MALE: INB22 | 20,499 | 928 | 95% |
| INB25 | 21,381 | 8600 | 60% |
| INB26 | 21,088 | 2652 | 87% |
| INB23 | 21,196 | 5615 | 74% |
| INB24 | 20,719 | 5825 | 72% |
| INB50 | 21,303 | 2389 | 89% |
| INB40 | 21,044 | 7809 | 63% |



Expectation

## III. 3. Hybrid MMP highly similar to Pericarp:

GBS Results

| Hybrid: Pericarp sample (GBS Only) | Endosperm Sample # | Total overlap calls | MMP Mismatches | Pericap & Hyb MMP % Sim |
|---|---|---|---|---|
| DK100-P-1 | DK100-E-12 | 671 | 8 | 99% |
| DK200-P-3 (source 1) | DK200-E-14 (source 1) | 618 | 9 | 99% |
| DK200-P-5 (source 2) | DK200-E-16 (source 2) | 455 | 3 | 99% |
| DK200-P-11 (source 2) | DK200-E-16 (source 2) | 676 | 8 | 99% |
| DK400-P-4 | DK400-E-15 | 452 | 34 | 92% |
| DK500-P-6 | DK500-E-17 | 536 | 16 | 97% |
| DK600-P-7 | DK600-E-18 | 515 | 16 | 97% |
| DK700-P-8 | DK700-E-19 | 581 | 8 | 99% |
| DK800-P-9 | DK800-E-20 | 559 | 12 | 98% |
| AVERAGE Comparison: Hybrid MMP to Pericarp | | 563 | 13 | 98% |

## IV. Summary: Hybrid Monomorphic Profile Results

GBS

| Comparison Between | Total Calls in Comparison | Average Similarity |
|---|---|---|
| MMP to FEMALE | 658 | 99% |
| MMP to MALE | 649 | 95% |
| MMP to Pericarp | 563 | 98% |

FP

| Match inbreds to MMP of DK200-E-Source 2 | Total Overlap with MMP | % Sim to MMP |
|---|---|---|
| FEMALE: INB21 | 21,091 | 99% |
| MALE: INB22 | 20,499 | 95% |

Fulfilled Expectation

- The MMP is highly similar to the Female and Pericarp genotypes
- The MMP is sufficiently highly similar to the male genotype.

## Conclusions from these experiments

1. Genotyping the hybrid's pericarp enables genotyping the female parent of a hybrid.
2. Pericarp DNA extraction requires large efforts to eliminate contamination from endosperm. SNP detection methods are limited
3. Utilizing a Hybrid Monomorphic Profile is a efficient method to capture both inbred parents similarity to hybrid.
4. Both methods of genotyping are good resources for finding the parental identity of an F1 hybrid.
5. MMP method is cost efficient and can be done in large scale. It can be supplemented with pericarp genotyping for further investigative work.

Note: For broader conclusions, an experimental design utilizing a larger spectrum of publicly available inbreds and their hybrids is recommended

## QESTIONS?

Gracias! Thank You! Obrigada!

[End of Annex and of document]