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GENEVA

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

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SURVEILLANCE: THREE APPROACHES TO USING SNPS (SINGLE NUCLEOTIDE POLYMORPHISMS) TO IDENTIFY VARIETY (INBRED LINE) USAGE

Document prepared by experts from Pioneer

Surveillance: Three approaches to using SNPs (single nucleotide polymorphisms) to identify variety (inbred line) usage

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Variety Surveillance

- Without surveillance and enforcement, why file for PVP protection?
- Program based on DNA markers (now SNPs), but previously used RFLPs and SSRs.
  - Minimize G X E interactions and subjectivity of morphology observations
  - Identity of parental inbred lines “obscured” by heterosis (hybrid vigor)

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Variety Surveillance

- Competitor hybrids purchased
- Profiled with 1536 SNPs (proprietary and public) or subsets of 768, 40, or 16 SNPs depending on analysis approach
  - Material profiled with replicated known standards to assess data quality
- Data analyses
  - Three approaches

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Approaches to Surveillance

1. Variety Similarity
   - Essentially Derived Variety (EDV)
2. Use of specific inbred lines via Pedigree Statistics analysis
   - Algorithm that selects most likely parentage
3. Use of specific inbred lines via a specially selected, highly discriminative set of SNPs
   - Algorithm used to select a small number of SNPs (<50) that will uniquely identify backgrounds of subject germplasm

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1. Variety Similarity

- Obtaining parental profiles
  - Pericarp inherited from female parent
  - Male SNP profile = Hybrid SNP profile – Female SNP profile

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ISF EDV DEFINITION –SSR

http://www.worldseed.org/cms/medias/file/Rules/EssentialDerivation/Guidelines_EDV_Maize_Lines_2008.pdf

- Based on marker similarity
- 3 level threshold
  - <82% -GREEN ZONE –Not at EDV
  - 82%-90% -ORANGE ZONE –Burden shifts to breeder of putative EDV
  - >=90% -RED ZONE –Strong indication of predominant derivation
- Functional SSR and SNP threshold translation estimate based on US and EU PVP inbreds w/public SSRs and SNPs

SSR threshold % similarity	SNP threshold % similarity
82 →	87-90
90 →	93-94

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1. Variety Similarity -Results

- Northern Hemisphere Region:
  - 8 competitor hybrids have pericarp similarities (87-91%) to another proprietary inbred line
  - 4 competitor hybrids have deduced male similarities (85-88%) to another proprietary inbred line
- Southern Hemisphere Region:
  - 11 competitor hybrids have similarities (99.2-99.8%) to another proprietary hybrid
  - 1 competitor hybrid with pericarp similarity (95%) to another proprietary inbred line

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Project to develop new maize EDV thresholds using SNPs

- Collaboration between ASTA (American Seed Trade Association) and UFS (l'Union Française des Semenciers)
- Examining widely used germplasm pools from US an EU
- Industry members added specific pairs of inbreds that are highly related (off-PVP) to address the EDV zones
- Profiled with Illumina 56,000 public SNP chip
- Post-doc at INRA currently analyzing the data
- Questions being addressed:
  - How many SNPs need to be analyzed to help determine EDV status?
  - What are the selection criteria for SNPs (genome coverage and informativeness)?

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2. Use of specific inbred lines - Pedigree statistics analysis

- Pedigree Statistics (Berry, et al., 2002, Jones et al., submitted to TAG)
  - Berry, D.A., J.D. Seltzer, C. Xie, D.L. Wright and J.S.C. Smith, 2002. Assessing probability of ancestry using simple sequence repeat profiles: applications to maize hybrids and inbreds. Genetics **161**:813-824.
  - Jones, E.S., S. Wall, D. Berry, D. Wright, and J.S.C. Smith. 2011. Assessing the Probability of Parentage in Maize Hybrids and Inbreds using Single Nucleotide Polymorphisms. In Press.
  - Identifies relative likelihood of parentage from a reference collection
  - Rank orders the most likely parents of a hybrid

Hybrid	Inbred Ranked	Rank Order	Prob_PedStats
HYB1	INB1	1	1.00000000
HYB1	INB5	2	1.00000000
HYB1	INB6	3	0.00000000
HYB1	INB2	4	0.00000000
HYB1		5	0.00000000
HYB1		6	0.00000000
HYB1		7	0.00000000
HYB1		8	0.00000000
HYB1		9	0.00000000
HYB1		10	0.00000000

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3. Use of specific inbred lines – Specially selected, highly discriminative SNP set

- Developed a high throughput variety identification screening process
  - To manage cost and sample/results turn-around, a smaller SNP set was selected specifically to provide maximum discrimination among a particular set of germplasm
- Jones et al., (2010) found that 16 SNPs were sufficient to distinguish ~400 inbreds
  - Jones, L., S. Wall, B. Nelson, and J.S.C. Smith, 2010. Varietal Identification in Maize: Are Sixteen SNP markers Sufficient? BMT/12/15 Twelfth Session Ottawa, May 11-14, 2010. UPOV, Geneva, Switzerland

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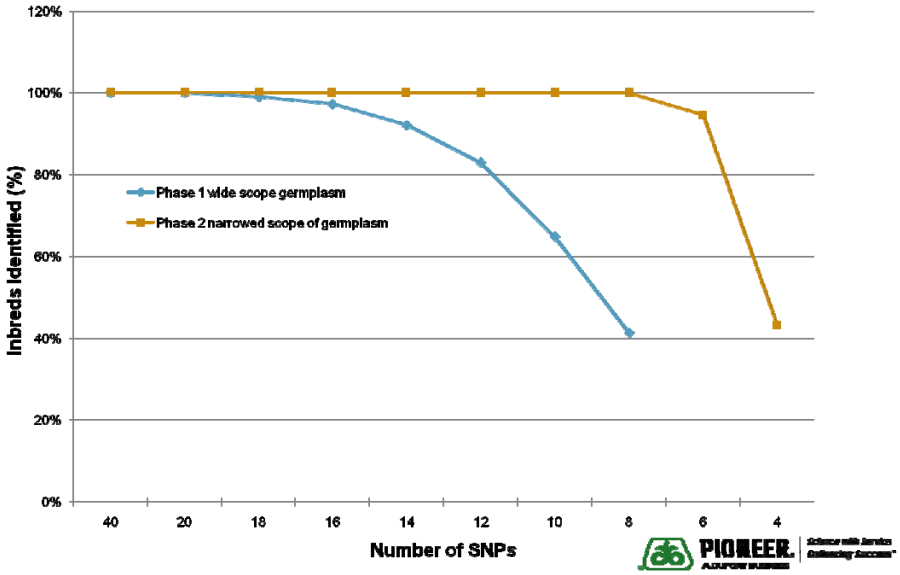
3. Use of specific inbred lines - Specially selected SNPs



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3. Use of specific inbred lines - Specially selected SNPs

- 40 SNPs selected
  - still a manageable number and gives extra redundancy
- Samples (leaf tissue and seeds) were interrogated with the 40 SNPs and compared to known germplasm profiles for identification

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3. Use of specific inbred lines - Specially selected SNPs

- To date: 52 samples have been analyzed identifying 18 instances of a specific proprietary inbred line or hybrid.
  - 18 misappropriations led to 8 lawsuits
  - 2700 hectares surveyed, 530 hectares contained misappropriated seed
    - Valued at ~$5 Million (US)

– Expect to analyze another 800 samples by year end.

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Summary

- Surveillance approaches using pedigree statistics and variety identification have identified several actual and other potential cases of misappropriation.
- Surveillance and enforcement are important components of ensuring effective Plant Breeders Rights.
- Enforcement of Plant Breeders Rights helps assure increased investments in, and generation of new more productive varieties for farmers, and ultimately for the benefit of consumers.

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