EDV:

The ISF approach and a case study to help determine EDV status

Oct. 2013
International Seed Federation

ISF consideration on essential derivation

- ISF strongly supports the concept of essential derivation
- Only few internationally agreed-upon professional rules
- Essential derivation is not a new right, but is in the scope of the right of a protected initial variety

Proof of predominant derivation

Various criteria or combination thereof:

- Phenotypic characteristics
- **Molecular characteristics**
- Breeding records
- Combining ability

Morphological vs. Molecular

- Distance Coefficients to define a threshold (trigger point for the reversal of the burden of proof)
- Geneticists and statisticians: technically equally possible to measure distance coefficients using morphological markers; but that these distances are not always reflective of genetic distances or of pedigree relationships.
- Use of morphological characteristics could be more difficult due to environmental factors, and much more expensive.
- ISF has mainly worked on thresholds (distances measured by molecular markers)

Requirement for EDV study

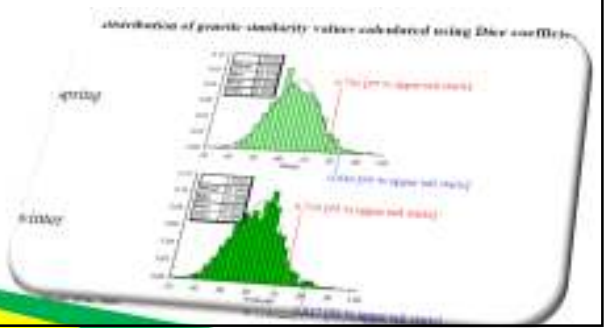
- Reference population
  To assess global genetic diversity
- EDV specific population
  To investigate potential EDV / non EDV cases
- Marker type
  To chose a tool
- Eligibility of molecular marker
  To define marker requirement
- Genetic similarities measurement
  To chose a genetic distance index

Choice of markers

- The markers must comply with several requirements:
  - Be “freely” available
  - Meet several technical criteria
    - =>addressed in an ISF document *“Issues to be addressed by technical experts to define molecular marker sets for establishing thresholds for ISF EDV arbitration”* (www.worldseed.org)

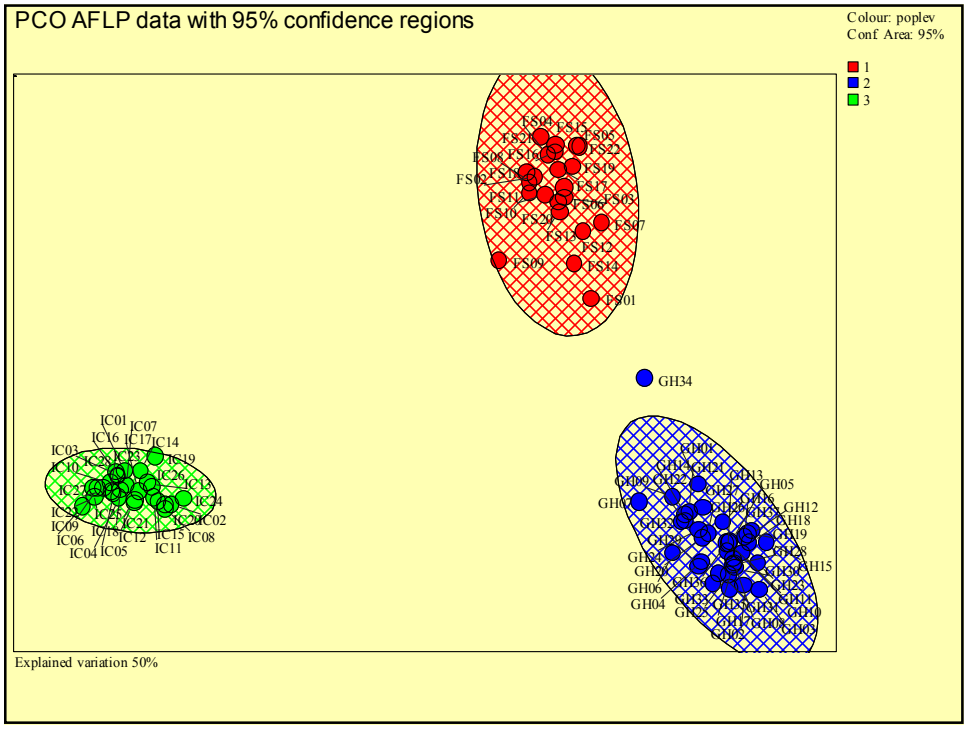
How to fix the threshold

- Use of pairs with known genealogy. Investigate genetic diversity : global vs closely related pairs
- Similarity exceeding a percentile point in the distribution of similarities (upper-tail approach)



Lettuce

- Three lettuce types: 35 GH, 21 FS & 27 IC
- Include most important varieties and maximum variation in each type.
- Varieties collected and shortlist made by ISF secretariat.
- In all ISF studies both varieties and companies are coded.



zone	q	genox	genoy	compx	compy	all	errormargin
2	0.9990	22	21	5	5	0.9985	0.0028
2	0.9973	19	15	4	4	0.9878	0.0079
2	0.9956	11	8	3	3	0.9850	0.0073
2	0.9939	13	11	3	3	0.9816	0.0093
2	0.9922	10	8	3	3	0.9759	0.0095
2	0.9906	35	30	6	6	0.9740	0.0159
2	0.9889	13	8	3	3	0.9717	0.0135
2	0.9872	27	25	5	5	0.9702	0.0132
2	0.9855	12	8	3	3	0.9686	0.0154
2	0.9838	11	10	3	3	0.9684	0.0120
2	0.9822	18	16	4	4	0.9671	0.0128
2	0.9805	35	31	6	6	0.9669	0.0109
2	0.9788	12	2	3	1	0.9667	0.0141
2	0.9771	31	30	6	6	0.9651	0.0107
2	0.9754	30	18	6	4	0.9651	0.0138
2	0.9738	13	12	3	3	0.9650	0.0158
2	0.9721	12	10	3	3	0.9639	0.0142
2	0.9704	20	16	4	4	0.9619	0.0108
2	0.9687	16	14	4	4	0.9618	0.0124
2	0.9670	12	11	3	3	0.9612	0.0129
2	0.9654	35	18	6	4	0.9593	0.0169
2	0.9637	8	2	3	1	0.9587	0.0160
2	0.9620	13	10	3	3	0.9582	0.0160
2	0.9603	33	18	6	4	0.9580	0.0196
2	0.9586	35	33	6	6	0.9579	0.0154
2	0.9570	30	14	6	4	0.9570	0.0159
2	0.9553	31	16	6	4	0.9565	0.0099
2	0.9536	27	24	5	5	0.9553	0.0154
2	0.9519	20	14	4	4	0.9538	0.0165
2	0.9502	17	15	4	4	0.9536	0.0185
1	0.9486	10	2	3	1	0.9534	0.0208
1	0.9469	33	31	6	6	0.9524	0.0201
1	0.9452	33	30	6	6	0.9519	0.0152
1	0.9435	31	29	6	6	0.9511	0.0208
1	0.9418	12	3	3	1	0.9507	0.0181
1	0.9402	35	16	6	4	0.9503	0.0146
1	0.9385	30	16	6	4	0.9501	0.0161

GH, Jaccard

GH21 and GH22 were selected in the same F4

GH8 and GH11 come from same F3

GH30 and GH35 come from same F3

GH27 is from a cross involving GH25

Lettuce

- 2004: 0.96 Jaccard similarity for all 3 cultigroups
- Trigger to initiate discussions => amicable settlement => arbitration => court

Oilseed Rape

- 4 Studies carried out between 2001-2006
- Bulks of 40 plants have a very high repeatability
- Bulks of 40 plants lead to a clear separation of all the varieties

- 2007: Dice dist. of 0.85 is trigger to start discussions (assessment according to protocol)

Ryegrass

- SSR's
- Guidelines
- Apply to all varieties (retroactive)
- Arbitration or court possible
- 1st Phase: Bulks provide same result as ind. Plants
- 2nd Phase: analyse variability in current varieties => come to threshold.
- Jaccard 0.6 reversal of burden of proof

Cotton

- Literature review on mol. mrkrs in cotton
- Gen. Div. within allotetraploid cotton varieties => no EDV threshold assigned
- Different approach: parentage
- 2007: If phen. or gen. char's suggest that 2 or more BC's were used or coefficient of parentage value is >87.5% => put. EDV
- Threshold is trigger point for discussions
- No settlement => arbitration

Tomato

To detect the use of a proprietary inbred line in a hybrid

- Phase 1 (2006 – 2007)
  - Daniela type
  - 21 hybrids, 35 parental lines from 5 companies
  - 93 SSR markers
- Phase 2 (2008 – 2009)
  - Cherry type
  - 17 hybrids, 34 parental lines from 6 companies
  - Same set of markers as for Daniela type
- Phases 3 and 4 (2010 – 2011)
  - SSR vs SNP markers
  - Different indices of similarity - Dice, Rogers and Simple Matching Percentage

Tomato

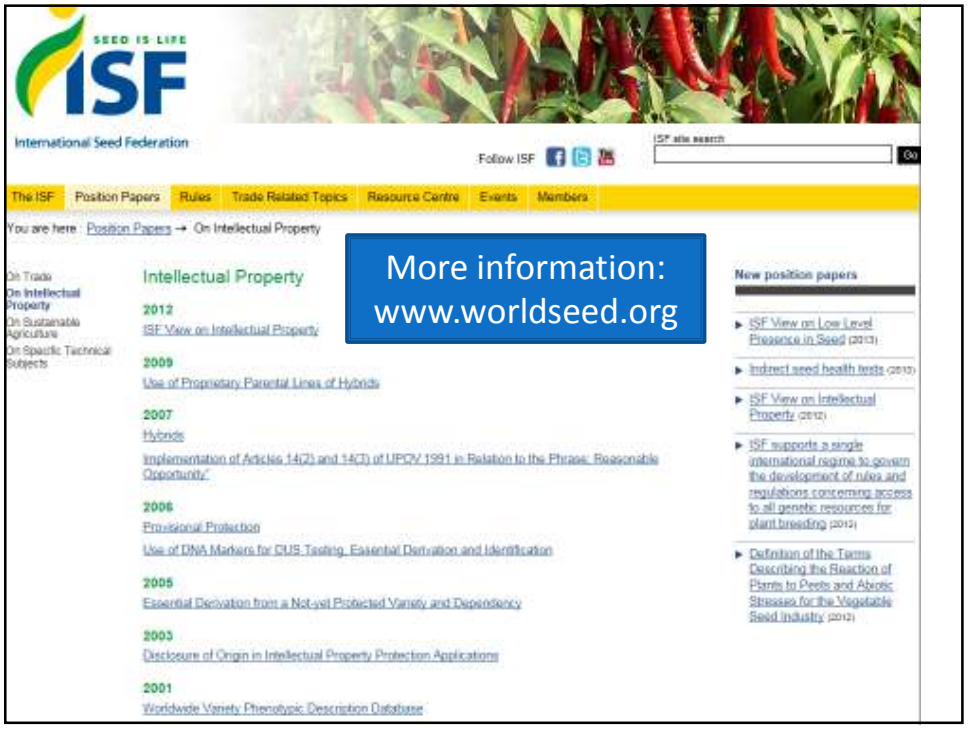
Conclusions

- SNP markers (7720 publicly available markers in the SOLCAP 10K Array) deliver data of excellent quality to detect the use of proprietary inbred line as a parent
- Compared to SSR, SNP markers are less sensitive when used with inbred lines that are were not fixed or had some residual heterozygosity
- A threshold for the Simple Matching Percentage (SM%) could be set to a value as high as 99.95
  - SM% - an index of genetic similarity that measures the match between alleles in the hybrid and the inbred line

ISF recommends to its members

In case of dispute:

- First enter into a conciliation or mediation procedure
- If that does not provide satisfactory results, enter into (binding) arbitration
- According to ISF Procedure Rules for Dispute settlement.



Maize

- 150 SSR markers, highly polymorphic
- Uniformly distributed, 80% coverage
- Avg 2 mrkrs/bin, Distance > 5cM
- Min. 3 alleles/mrkr, PIC min 0.3 [0.6-0.7]

- 2008: At 82% conformity: burden of proof shifts to breeder of put. EDV
- At 90% conformity: strong indication of predominant derivation

Molecular Marker Data: Criteria

- Molecular markers provide a genetic measure of relatedness
- Methodology must be scientifically sound and readily available
  - Highly discriminative, repeatable, readily scorable
  - Data analysis, statistically sound,
  - Meet peer reviewed standards for publication
  - Be publicly available including at third party laboratories

Agreed interpretation of the data

- Genetic similarity-zone thresholds
- Meaning of thresholds-zones: determining predominant derivation e.g.
  - Red zone: greater certainty of predominant derivation
  - Orange zone: possible predominant derivation
  - Green zone: no evidence of predominant derivation
  - Red or orange would reverse the burden of proof
  - More precise interpretations of red and orange zones as evidence of predominant derivation may also be adopted

Joint multi-company International Approaches: SSRs

- Early 2000s, ASTA, SEPROMA, and experts from Germany (Univ. of Hohenheim) examined SSR data:
- 1) US inbreds of known pedigree (unrelated-99% similar)
- 2) Inbreds PVP'd in Europe of (largely) unknown pedigree, but of known performance
- 3) computer modeling simulation studies

Joint multi-company International Approaches: Agreed upon thresholds using SSRs

- Below 82% SSR similarity: Green Zone
  - No predominant derivation
- 82%-89% SSR similarity: Orange zone
  - "the burden of proof shifts to the breeder of the putative essentially derived variety. Other criteria should be evaluated including combining ability, phenotypic characteristics and breeding records " (ISF, 2008)
- 90% or above: Red zone
  - "a strong indication of predominant derivation."

ASTA / UFS - SNP project

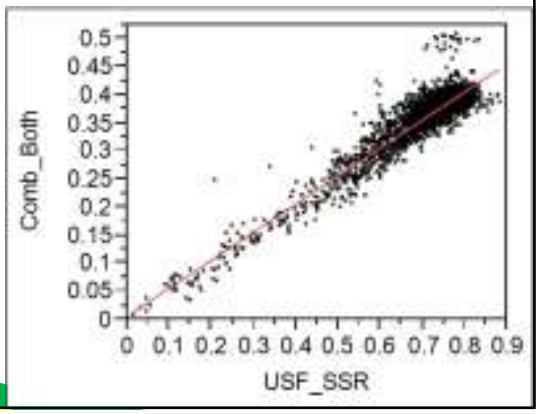
- Materials:
  - Inbred lines and SSRs used to establish SSR thresholds.
  - 26,784 SNPs from 50k snp chip (dropped those that performed poorly, or were biased toward showing differences between only B73 and Mo17)
- Goals:
  - Compare genetic similarities between inbred lines using SNP and SSR data
  - Determine number of SNPs needed
  - Convert SSR based threshold-zones to their SNP equivalents
- Essentially a recalibration exercise

SNPs selected using the genetic map => correlation 0.92

Subsequent analyses using
* half of SNPs using genetic map &
* half of SNP's using physical map,
[ensures more thorough and comprehensive sampling of genome]
=> higher correlation of 0.94

Based on a set of SNP's optimized and selected jointly from the genetic map and the physical map

Correlation SSR-SNP
R square 0.94
Slope = 0.5"



DRAFT Proposals

- Use a mix of markers based upon physical and genetic maps
- 1536 or 3072 SNPs
  - SD 0.006 and 0.004, respectively
- 95% + = red zone
- 91%-94% = orange zone
- <90% = green zone
- Final tests of SNP sets underway
- Next steps
  - Present to ASTA, UFS Boards, ISF, write manuscript for peer review and publication in the scientific literature

DRAFT Proposals

- 1536 or 3072 SNPs markers chosen based upon physical and genetic maps
  - SD 0.006 and 0.004, respectively
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- Proposed equivalent threshold
  - 95% + = red zone
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  - <90% = green zone
- Next steps
  - Present to ASTA, UFS and ISF for validation, write manuscript for peer review and publication in the scientific literature

References

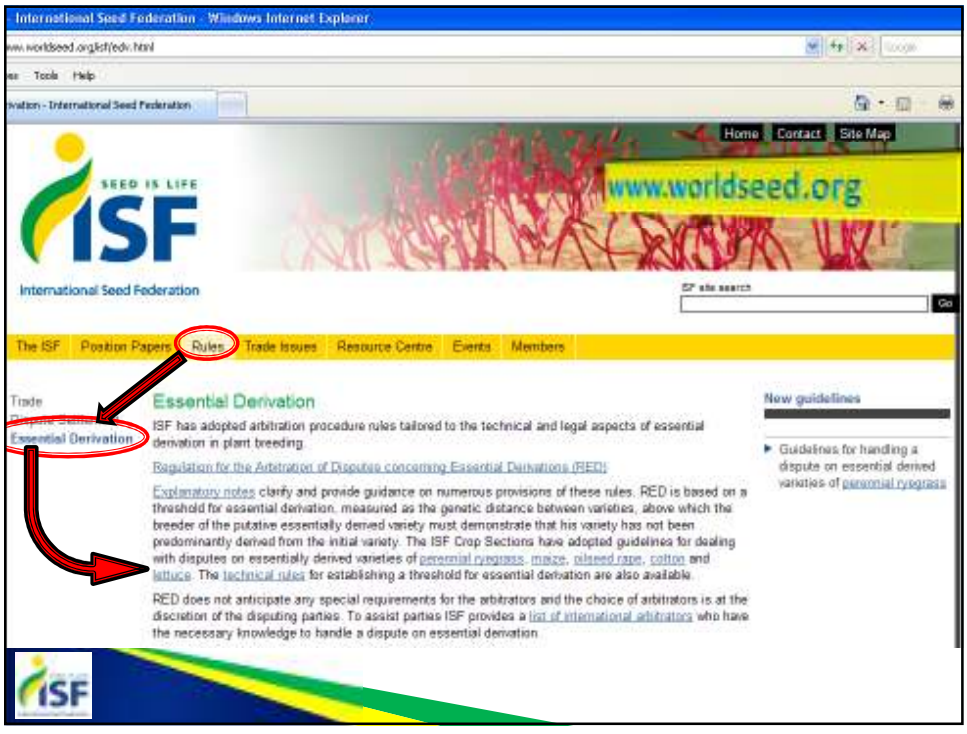
- F. Van Eeuwijk et al., Statistical aspects of essential derivation, with illustration based on lettuce and barley. 2004. Euphytica 137: 129
- Heckenberger et al., Identifying Essentially derived Varieties with molecular markers. 2005. T.A.G. 111: 598
- Smith el al., Genetic Diversity among U.S. Sunflower Inbreds and Hybrids: Assessing Probability of Ancestry and Potential for Use in Plant Variety Protection. 2009. Crop Sci 49: 1295

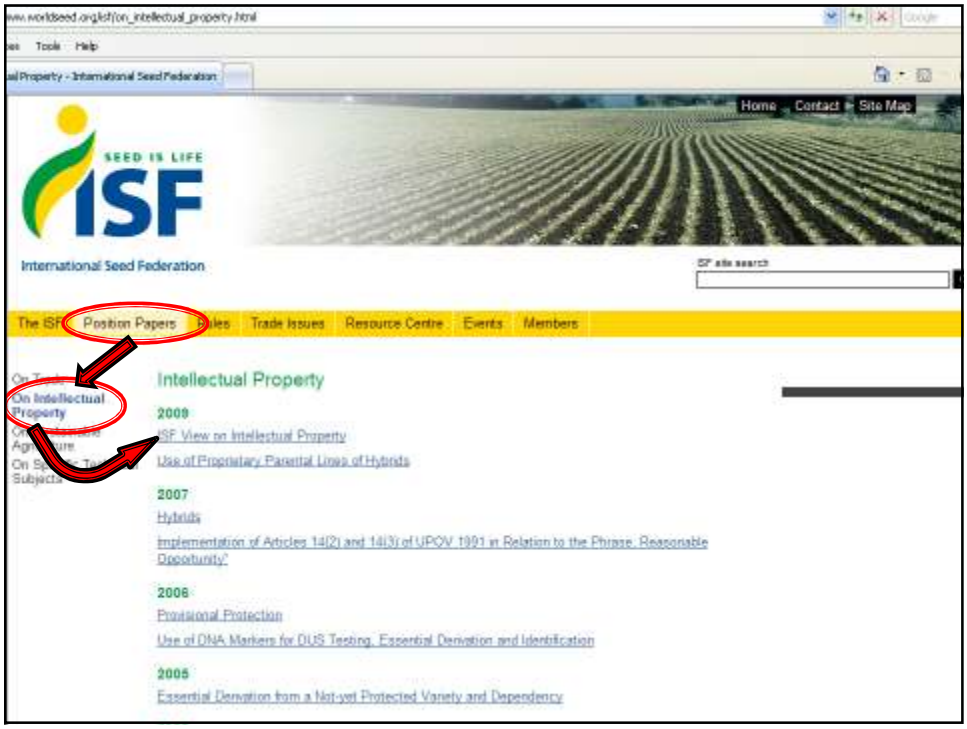
Maize EDV Case Study

- DUS are NOT the issue
- Predominant derivation IS the issue
- Pedigree IS the issue
- Closeness of pedigree or genetic similarity IS the issue
- Prima facie evidence is initially difficult to obtain:
  - Lots of resource, time demanding morphological comparisons
  - BUT morphology often obscures genetic-pedigree similarity
  - Pedigree breeder notes confidential
  - Impossible to morphologically compare proprietary parental lines
- Molecular marker data can provide a genetic measure

ASTA / UFS - SNP project

- SNPs replacing SSRs
- Scientific team
  - Post-doc Yves Rousselle
  - INRA Plant Genetics group, Alain Charcosset, France
  - Rex Bernardo - Univ Minnesota USA
  - Benjamin Stich - Max Planck Institute Germany
- ASTA and UFS company representatives
  - Agreliant, Caussade Semences, Dow, Euralis, KWS, Limagrain, Maïsadour, Monsanto, DuPont Pioneer, RAGT, Syngenta,

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