



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



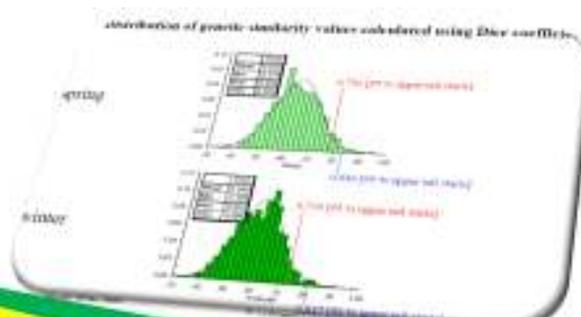
Choice of markers

- 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

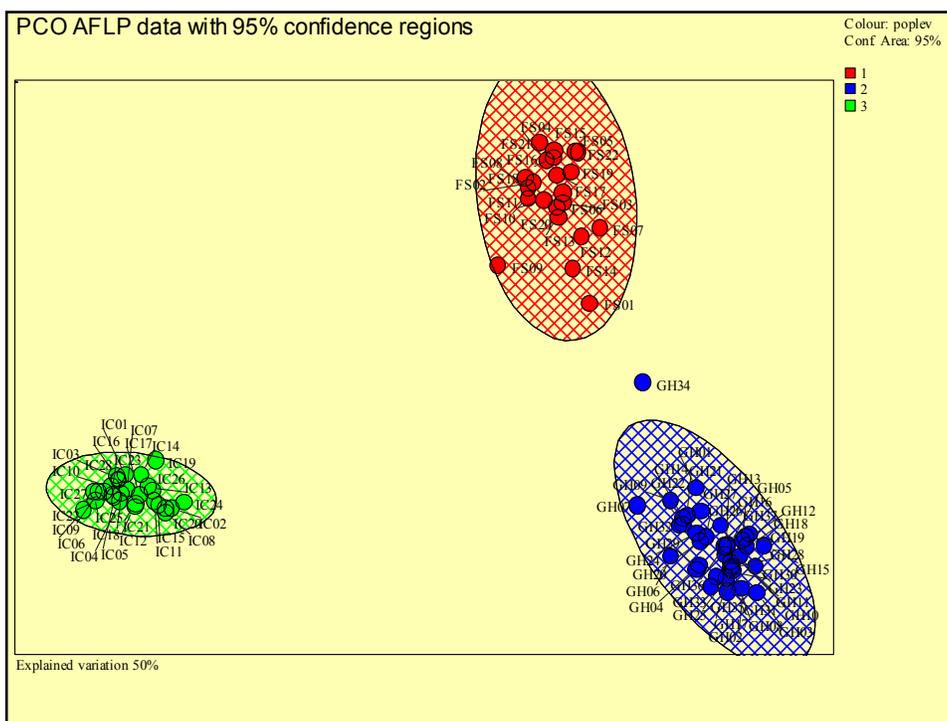
- Use of pairs with known genealogy.
Investigate genetic diversity : global vs closely related pairs
- Similarity exceeding a percentile point in the distribution of similarities (upper-tail approach)



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	an	enormargin
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
- Bults of 40 plants have a very high repeatability
- Bults 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 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.



The screenshot shows the ISF website interface. At the top left is the ISF logo with the tagline "SEED IS LIFE". Below it, the text "International Seed Federation" is visible. A navigation menu includes "The ISF", "Position Papers", "Rules", "Trade Related Topics", "Resource Centre", "Events", and "Members". A search bar is located on the right. The main content area is titled "Intellectual Property" and lists various position papers from 2001 to 2012. A blue box with white text is overlaid on the page, stating "More information: www.worldseed.org".

**More information:
www.worldseed.org**

Intellectual Property

- 2012**
[ISF View on Intellectual Property](#)
- 2009**
[Use of Proprietary Parental Lines of Hybrids](#)
- 2007**
[Hybrids](#)
[Implementation of Articles 14\(2\) and 14\(3\) of UPOV 1991 in Relation to the Phrase: Reasonable Opportunity](#)
- 2006**
[Emissional Protection](#)
[Use of DNA Markers for QUS Testing, Essential Derivation and Identification](#)
- 2005**
[Essential Derivation from a Not-yet Protected Variety and Dependency](#)
- 2003**
[Disclosure of Origin in Intellectual Property Protection Applications](#)
- 2001**
[Worldwide Variety Phenotypic Description Database](#)

New position papers

- ▶ [ISF View on Low Level Presence in Seed \(2013\)](#)
- ▶ [Indirect seed health tests \(2013\)](#)
- ▶ [ISF View on Intellectual Property \(2012\)](#)
- ▶ [ISF supports a single international regime to govern the development of rules and regulations concerning access to all genetic resources for plant breeding \(2012\)](#)
- ▶ [Definition of the Terms Describing the Reaction of Plants to Pests and Abiotic Stresses for the Vegetable Seed Industry \(2012\)](#)

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, SEPRONA, 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]

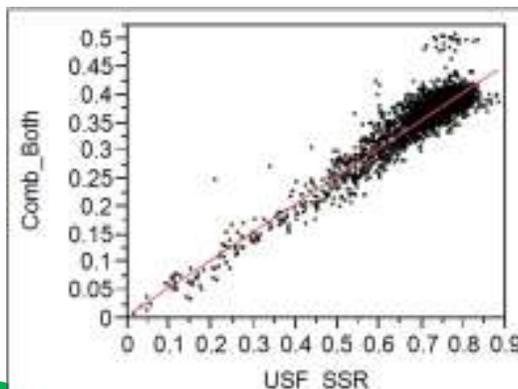
=> higher correlation of 0.94

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
 - Final tests of SNP sets underway
- ▶ Proposed equivalent threshold
 - 95% + = red zone
 - 91%-94% = orange zone
 - <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 et 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





Thank you for your attention



SEED IS LIFE
ISF
International Seed Federation

More information:
www.worldseed.org

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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, Maisadour, Monsanto, DuPont Pioneer, RAGT, Syngenta,



International Seed Federation - Windows Internet Explorer

www.worldseed.org/isd/educ.html

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Trade

Essential Derivation

ISF has adopted arbitration procedure rules tailored to the technical and legal aspects of essential derivation in plant breeding.

[Regulation for the Arbitration of Disputes concerning Essential Derivations \(RED\)](#)

Explanatory notes clarify and provide guidance on numerous provisions of these rules. RED is based on a threshold for essential derivation, measured as the genetic distance between varieties, above which the breeder of the putative essentially derived variety must demonstrate that his variety has not been predominantly derived from the initial variety. The ISF Crop Sections have adopted guidelines for dealing with disputes on essentially derived varieties of [perennial ryegrass](#), [maize](#), [oilseed rape](#), [cotton](#) and [lettuce](#). The [technical rules](#) for establishing a threshold for essential derivation are also available.

RED does not anticipate any special requirements for the arbitrators and the choice of arbitrators is at the discretion of the disputing parties. To assist parties ISF provides a [list of international arbitrators](#) who have the necessary knowledge to handle a dispute on essential derivation.

New guidelines

▶ Guidelines for handling a dispute on essential derived varieties of [perennial ryegrass](#)

The screenshot shows a web browser window displaying the ISF website. The address bar shows 'www.worldseed.org/ifa/ifa_intellectual_property.html'. The page features the ISF logo with the tagline 'SEED IS LIFE' and 'International Seed Federation'. A navigation menu includes 'The ISF', 'Position Papers', 'Policies', 'Trade Issues', 'Resource Centre', 'Events', and 'Members'. The 'Position Papers' menu item is circled in red, with two red arrows pointing to the 'Intellectual Property' section of the page. This section lists several papers with their years and titles:

- 2009**
 - [ISF View on Intellectual Property](#)
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