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UPOV

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INTERNATIONAL UNION FOR THE PROTECTION OF NEW VARIETIES OF PLANTS
GENEVA

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

Twelfth Session
Ottawa, Canada, May 11 to 13, 2010

EDV – THE ISF APPROACH

Document prepared by experts from the International Seed Federation (ISF)

EDV – The ISF approach

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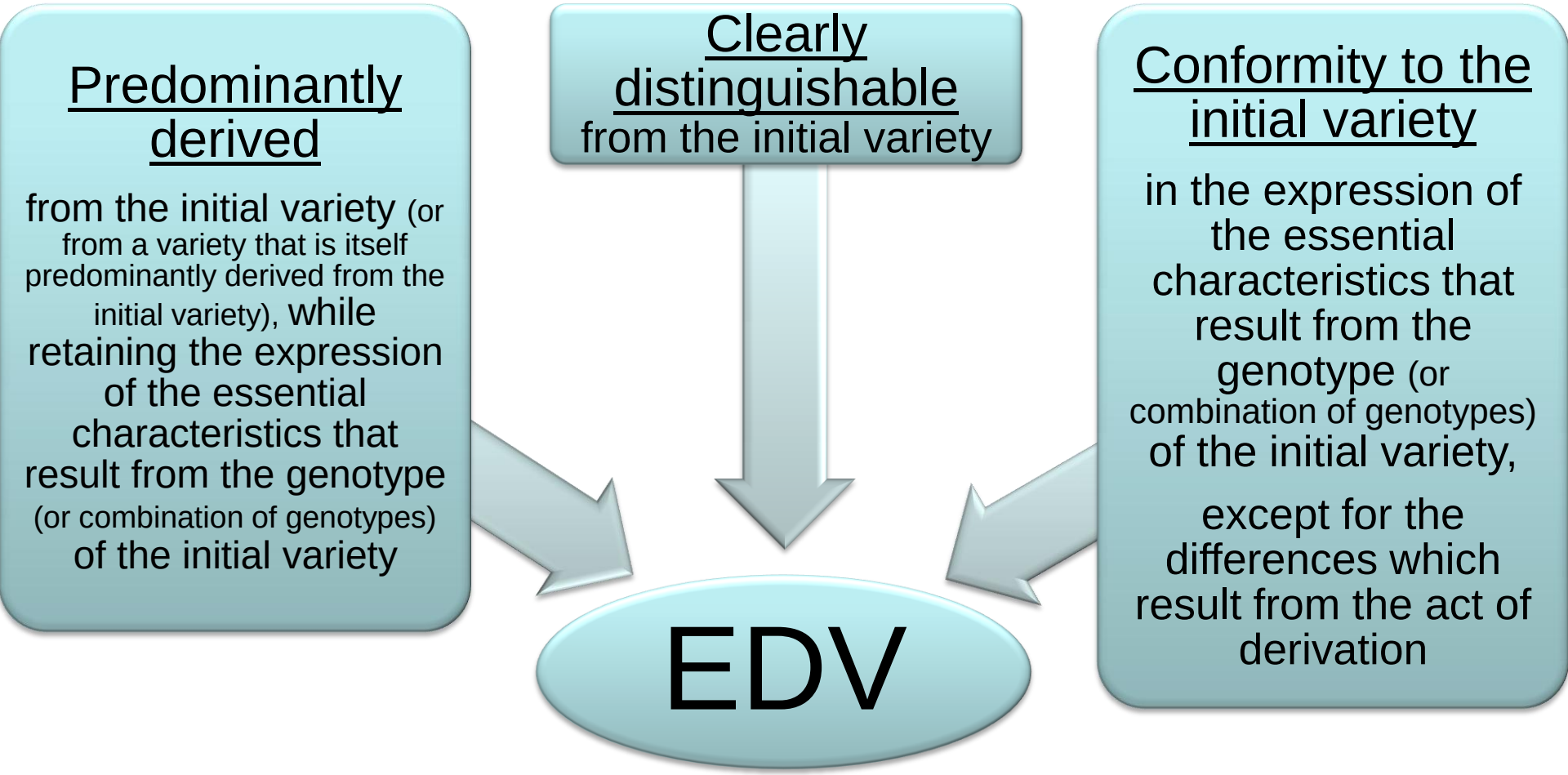
Ottawa, May 2010

Outline

1. Essentially Derived Varieties in the UPOV Convention
2. ISF consideration on essential derivation
3. Assessment of essential derivation
4. Burden of Proof
5. Use of molecular markers, a crop-by-crop approach:
    i. Lettuce
    ii. Oilseed Rape
    iii. Ryegrass
    iv. Cotton
    v. Tomato
    vi. Maize

1. Essentially Derived Varieties in the UPOV 1991 Convention



2. 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

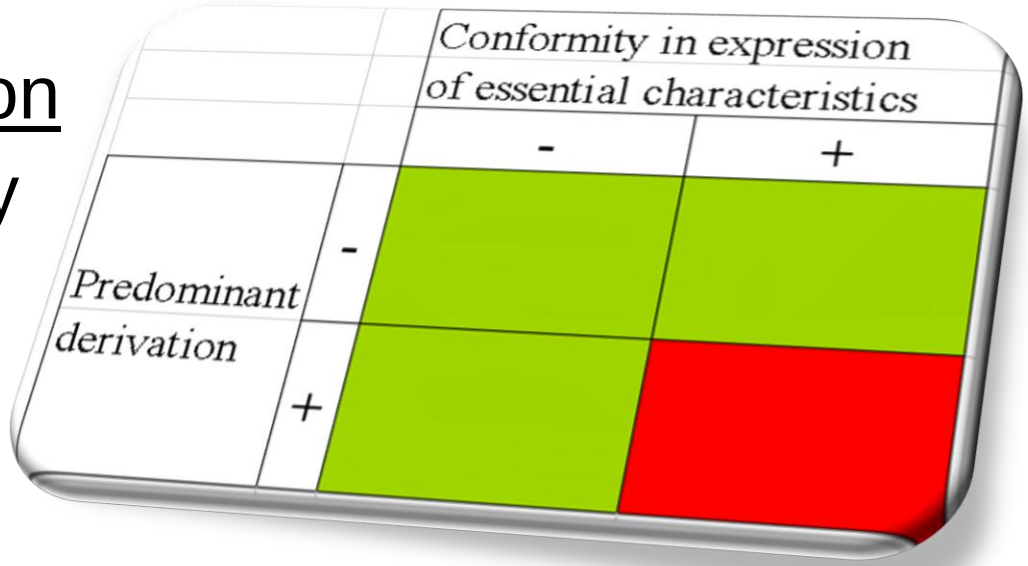
3. Assessment of essential derivation

After establishing that the variety is <u>distinct</u> (DUS)

=> consider the following requirements:

- <u>Conformity</u> to the initial variety in the expression of the essential characteristics that result from the genotype or the combination of genotypes of the initial variety
- <u>Predominant derivation</u>

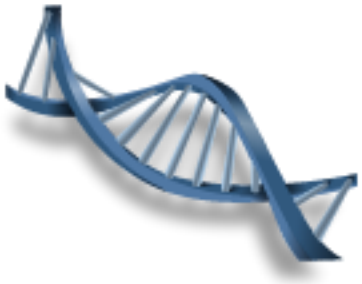
  from the initial variety



Proof of predominant derivation

Various criteria or combination thereof:

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



4. Burden of proof

For « prima facie » proof, the following elements should be sufficient:

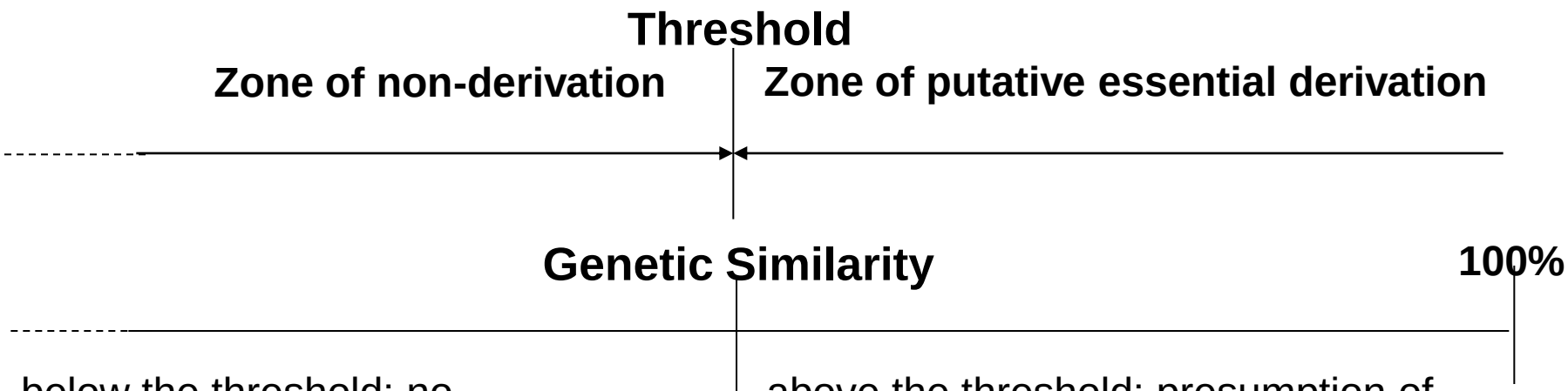
- Strong phenotypic similarity
- Only small differences in some simply inherited characteristics
- **Strong genetic similarity**

If the owner of the i.v. has fulfilled one of the above requirements, then the second breeder would have to prove that there is no predominant derivation, or that he had not used the i.v., or a variety essentially derived from that i.v.

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)
- .

Threshold: divide the scale of conformity into two parts



below the threshold: no presumption of essential derivation

above the threshold: presumption of essential derivation and the burden of proof of non predominant derivation would fall on the breeder of the putative EDV

Threshold will vary from species to species, depending on the existing genetic variability within the species and the established breeding procedures.

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.

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5. **Use of molecular markers**, a crop-by-crop approach:
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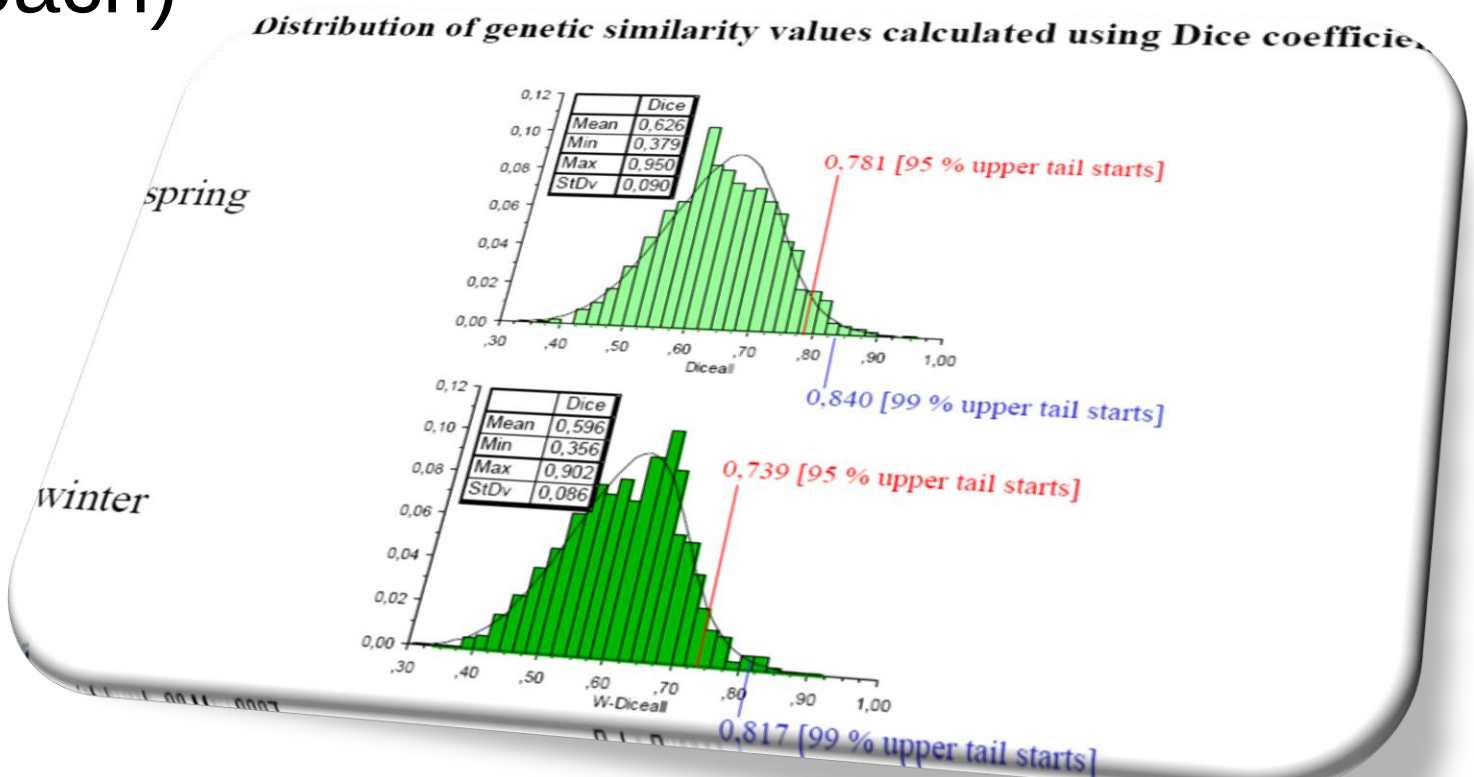
Choice of markers

- 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
- Similarity exceeding a percentile point in the distribution of similarities (upper-tail approach)



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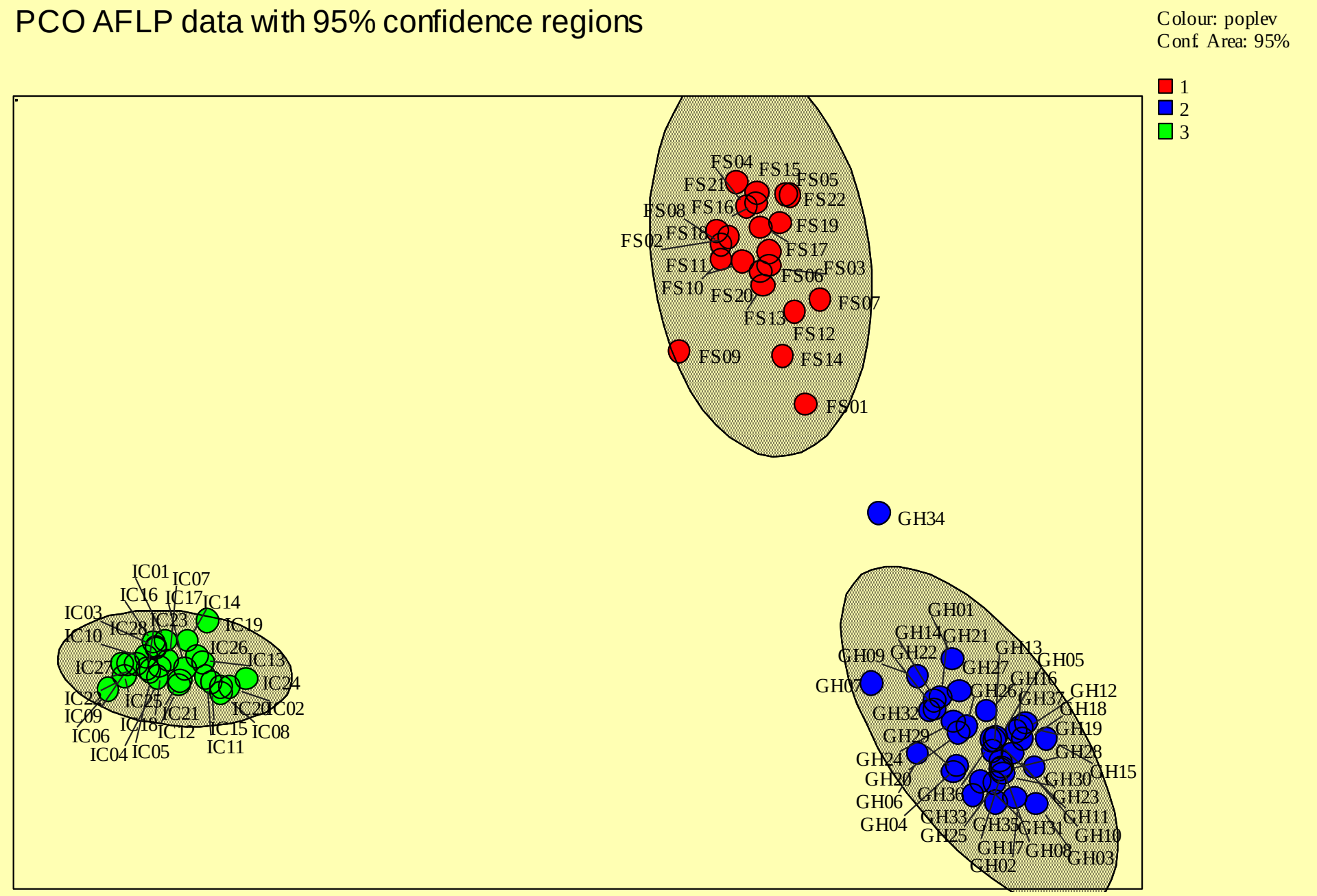
Lettuce EDV study

- Three lettuce types: 35 GH, 21 FS & 27 IC
- Long-list made to 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.


PCO AFLP data with 95% confidence regions
Colour: poplev
Conf. Area: 95%
1
2
3
FS04
FS15
FS21
FS05
FS22
FS08
FS16
FS19
FS18
FS02
FS17
FS11
FS03
FS06
FS10
FS20
FS07
FS13
FS12
FS09
FS14
FS01
GH34
IC01
IC07
IC16
IC17
IC14
IC03
IC28
IC23
IC19
IC10
IC26
IC13
IC27
IC24
IC22
IC25
IC21
IC20
IC02
IC09
IC18
IC12
IC15
IC08
IC06
IC04
IC05
IC11
GH01
GH14
GH21
GH13
GH09
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GH27
GH05
GH07
GH16
GH26
GH37
GH12
GH32
GH18
GH19
GH29
GH28
GH24
GH15
GH20
GH30
GH36
GH23
GH06
GH33
GH35
GH31
GH11
GH04
GH25
GH17
GH08
GH10
GH03
GH02
Explained variation 50%

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 EDV study

- 2004: 0.96 Jaccard similarity for all 3 cultigroups
- Trigger to initiate discussions => amicable settlement => arbitration => court
- Review in 5 years

Oilseed Rape Study

- 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)
- Review in 5 yrs

Ryegrass Study - 1

- 2002 Code of Conduct adopted
- 60 plants/variety, 5 primer comb.
- Squared Euclidean distance lower than 7⇒ ask for arbitration
- Apply only to varieties released after adoption
- 2004 concerns by members
- New study initiated

Ryegrass Study - 2

- New study SSR's (instead of AFLP's)
- Guidelines (instead of CoC)
- Apply to all varieties
- Court possible, not only arbitration
- 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

Ryegrass Study - 2

Guidelines for Handling a Dispute on Essential Derivation in Ryegrass

(Adopted by the ISF Forage and Turf Section, November 2009)

1. The 1991 Act of the UPOV Convention introduced the concepts of essent dependency from an initial variety (i.v.).
2. The Forage Plants Section of ASSINSEL conducted a study in 1997 and tools and to determine a possible threshold for assessing putative essential
3. Based on the results of that study "Principles of a Code of Conduct in Es Varieties of Perennial Ryegrass" were adopted by the Section in 2002. Thes replace that Code of Conduct.
4. In 2005 the ISF Forage and Turf Crops Section decided to conduct a new new testing protocol using SSR markers on bulked plants instead of using individual plants.
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   d. If an
      i.

TECHNICAL·PROTOCOL·FOR·ASSESSMENT·OF·GENETIC·DISTANCE·FOR·ISF·GUIDELINES·FOR·THE·HANDLING·OF·A·DISPUTE·ON·EDV·IN·DIPLOID·PERENNIAL·RYEGRASS¶

Microsatellite· Marker·Analysis· –·Experimental· procedures¶

1. → Sampling·of·leaf·material¶

¶

--→ Define·two·random·sub-samples·of·20·plants·/·variety·for·sampling.·These·two·sub-samples·are·analyzed·in·bulk.·Each·bulk·is·formed·by·a·different·set·of·plants·of·a·given·variety.¶

--→ Harvest·even-sized·leaf·segments·from·each·plant·that·contributes·to·a·given·bulk.·Young·leaves·should·be·used·for·sampling.·The·twenty·leaf·segments·that·constitute·

Table·1.·List·of·31·SSR·markers·with·fragment·size·range,·number·of·identified·alleles·and·allele·sizes·(based·on·the·sizes·determined·by·ILVO,·fragment·size·might·vary·based·on·the·used·platform·and·laboratory,·the·use·of·reference·samples·is·strongly·advised,·as·the·specific·sizing·of·the·fragments·is·system-·and·laboratory-dependent).·The·data·on·number·of·alleles·and·range·correspond·to·the·results·obtained·in·the·study·described·in·Roldán-Ruiz·et·al.·(in·preparation)¶

Original·locus·denomination	Original·primer·pair	New·code·name	New·primer·pair	#·alleles	Range·(bp)
B1A2	F:·GTGCAGCAGTTTGAATTGGA¶ R:·AGCATCGGGAGCTATGAATG	R01	F:·TTTGAATTGGATTGGTCTGGTT¶ R:·TCGGGAGCTATGAATGATGA	17	116-243
B1A8	F:·GACTTTCAGGCATCGGTCAT¶ R:·CCCAGCTCCATTCTTAATGC	R02	F:·TTGGAAGATGCCAAAGTGAAG¶ R:·GGAAGGCTCCATAATTCTCCTC	11	180-222
B1B3	F:·AGGTGTCCTGTTGCTTTGGA¶ R:·TTTACCCCCAGGGATCAAAT	R03	F:·TACAATCCTTGATCCTGTCTGC¶ R:·CCCAGGGATCAAATCACATAAA	8	291-403
B1C8	F:·TTCTGGCCATGTTGATTTGC¶ R:·GTCTACGGGTTGGAGCAGTG	R04	F:·GCAGGGTGAATTTGAAGCA¶ R:·TACGGGTTGGAGCAGTGG	4	177-184
B1C9	F:·GAGCCGATGCACAGGTTACT¶ R:·AAAGGAAGCCGGCTAATCAC	R05	F:·TCATCGGTCAGGTGTTTCCT¶ R:·GCCGGCTAATCACCAAAGT	8	132-168
B2G6b	F:·CCAACTAGACAAAGGGGATTG¶ R:·GGAGAGCACCATTCATCCAT	R07	F:·AGAATTCGGATCACAACCAACT¶ R:·GGATCTTGAAGGGCAACG	7	99-129
B3A1	F:·CTTGTCGTCCTTGTTGGGAG¶ R:·ATATTCTGGATCGTGGCGTT	R08	F:·TGCTACTCTTGTCGTCCTTGTT¶ R:·CTGGATCGTGGCGTTGTT	3	309-318
B3A3	F:·GGGTGAAGTGCTCTTTGTGA¶ R:·ATGGTGAAGGCCTGAAACTG	R09	F:·GGTAGGGTGAAGTGCTCTTTGT¶ R:·CAACCAGTGCTTCAGCTTTGT	4	352-363
B3C10	F:·CTACAACTCCGTGCTGCTGA¶ R:·TGCATGGTTTCTCAAATGCT	R12	F:·GCTGCTGATGCCAACCAT¶ R:·GCTCTCTAACACCATATCAACTCG	4	320-323
B3C5	F:·TGTCATGTTCAGAAAGTGCG¶ R:·TGTCCACATAAATGCACCTCA	R14	F:·TGGGAGCACAACATCTCG¶ R:·CACATAAATGCACCTCAGAACAA	13	190-213
B3D2	F:·ATACGAGCGAATTGCCTCTC¶ R:·TCTCCCATCGCTTATGTTCC	R16	F:·GGTACCAATAATCAGAGGCTAAACAA¶ R:·CGCCACCATTGATGAACTC	9	392-425
B3E6	F:·CTGTAACAACAGCCGCTGAG¶ R:·GTCTCGAGCACAGGAGTTCA	R18	F:·GATCAGCCAACAATCACTCG¶ R:·CGCAGCAACAGTCTAACGAT	4	350-354

Cotton Study

- 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

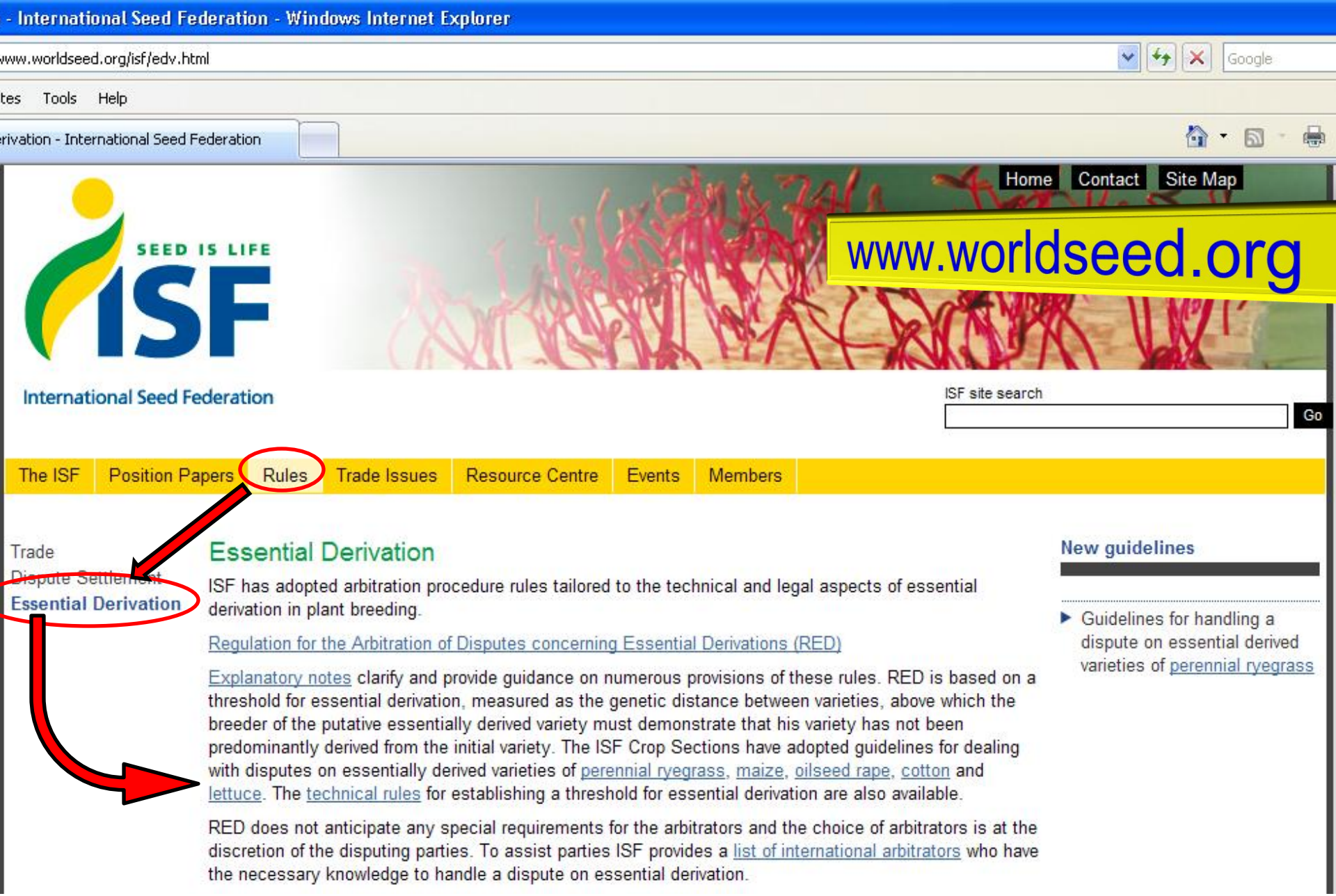
Tomato Study

- Started 2006, Daniella type
- 21 hybrids & 35 parent lines
- 93 SSR markers used for data analysis
- Dice coefficient of 0.78 between F1 and parent line : trigger point for suspected use of proprietary line in production of a hybrid.
- Continuation with cherry type => 0.80 Dice

Maize Study

- 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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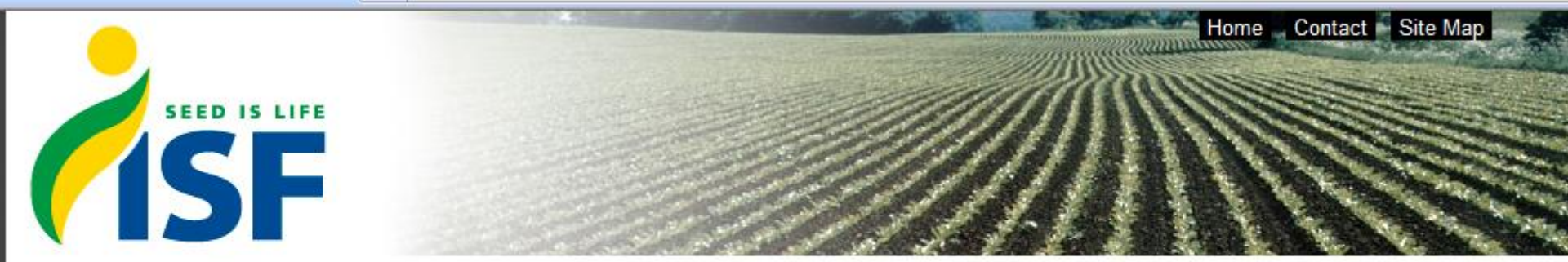
Essential Derivation
Essential Derivation
ISF has adopted arbitration procedure rules tailored to the technical and legal aspects of essential derivation in plant breeding.
Regulation for the Arbitration of Disputes concerning Essential Derivations (RED)
Explanatory notes clarify and provide guidance 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



Intellectual Property

2009

ISF View on Intellectual Property

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

Provisional Protection

Use of DNA Markers for DUS Testing, Essential Derivation and Identification

2005

Essential Derivation from a Not-yet Protected Variety and Dependency

Thank you for your attention

SEED IS LIFE
ISF
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