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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

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INTERNATIONAL SEED FEDERATION (ISF) OILSEED RAPE ESSENTIALLY DERIVED VARIETIES (EDV) STUDY

Document prepared by the International Seed Federation Working Group

ISF OILSEED RAPE EDV STUDY

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Composition of ISF Working Group

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Objective

- Is it possible to use molecular markers to define a genetic similarity threshold as a trigger to initiate a dispute settlement process in alleged case of essential derivation?

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Parameters

- Do we have suitable techniques?
- How to take into account the specific crops characteristics?

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Suitable Technique

- 80 SSR markers
- Mapped and well distributed
- Freely available or available against reasonable payment (80€ per marker)

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Specific Crop Characteristics

- Estimation of intra-varietal versus inter-varietal variability
- Using pooled samples to increase discrimination power
- Estimation of variation between different seed lots of certain varieties, in particular inter-annual variability

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First Phase

- estimation of intra-varietal versus inter-varietal variance

Plant material (15 varieties, 5 pop structures, 5-10 bulk samples/ variety, 5 plants/bulk)

Name	Type	Category	TG Code	Plants/bulk	Analysed bulks
Mikado	winter	DH	No. 02	5	5
Quantum	spring	DH	No. 08	5	10
Pollen	winter	DH	No. 13	5	10
Legend	spring	narrow pop	No. 03	5	5
Westar	spirng	narrow pop	No. 06	5	5
Lirajet	winter	narrow pop	No. 09	5	10
Columbus	winter	pure line	No. 01	5	5
Drakkar	spring	pure line	No. 04	5	5
Bristol	winter	pure line	No. 15	5	10
Dexter	winter	Synthetic	No. 10	5	10
Winner	winter	Synthetic	No. 11	5	10
Karola	winter	Synthetic	No. 12	5	10
Excel	spring	wide pop	No. 05	5	5
Navajo	winter	wide pop	No. 07	5	10
Rainbow	spring	wide pop	No. 14	5	10

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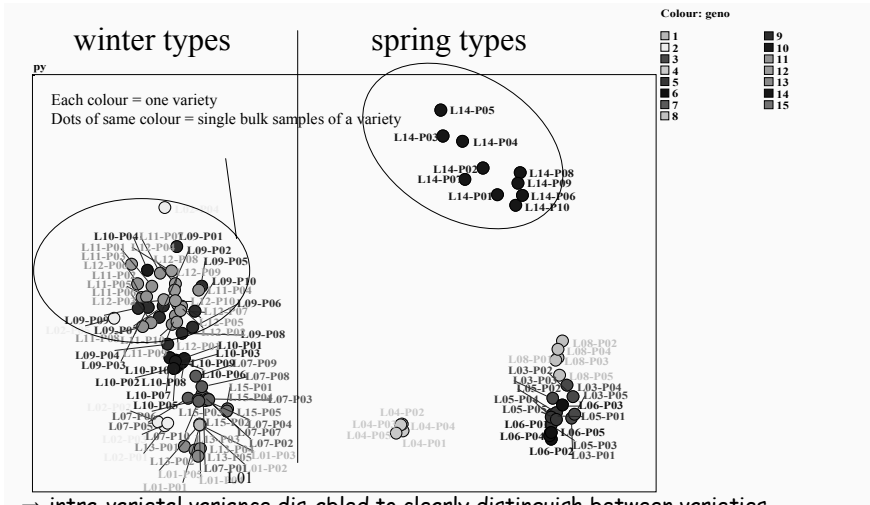
First Phase Results

- estimation of intra-varietal versus inter-varietal variance



⇒ intra-varietal variance dis-abled to clearly distinguish between varieties
⇒ mis-classification of single bulks increases with degree of heterogenity in a variety

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Conclusions of First Phase

- The SSR set demonstrates an acceptable level of quality and variability

=> we can continue with this set of markers

- Populations and synthetics, based on 5 plants per bulk, show a high degree of intra-varietal variation => difficult to distinguish varieties

=> would "bigger" pools give a better discrimination?

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Second Phase

b) using pooled samples to increase discrimination power

Plant material (15 varieties, 5 pop structures, 2 bulk samples/ variety, 40 plants/bulk)

				ISF TG Analysis 1		ISF TG Analysis 2	
Name	**Type**	**Category**	**TG Code**	**Plants/bulk**	**No bulks**	**Plants/bulk**	**No bulks**
Mikado	winter	DH	No. 02	5	5	40	2
Quantum	spring	DH	No. 08	5	10	40	2
Pollen	winter	DH	No. 13	5	10	40	2
Legend	spring	narrow pop	No. 03	5	5	40	2
Westar	spirng	narrow pop	No. 06	5	5	40	2
Lirajet	winter	narrow pop	No. 09	5	10	40	2
Columbus	winter	pure line	No. 01	5	5	40	2
Drakkar	spring	pure line	No. 04	5	5	40	2
Bristol	winter	pure line	No. 15	5	10	40	2
Dexter	winter	Synthetic	No. 10	5	10	40	2
Winner	winter	Synthetic	No. 11	5	10	40	2
Karola	winter	Synthetic	No. 12	5	10	40	2
Excel	spring	wide pop	No. 05	5	5	40	2
Navajo	winter	wide pop	No. 07	5	10	40	2
Rainbow	spring	wide pop	No. 14	5	10	40	2
15 varieties					120 samples		30 samples

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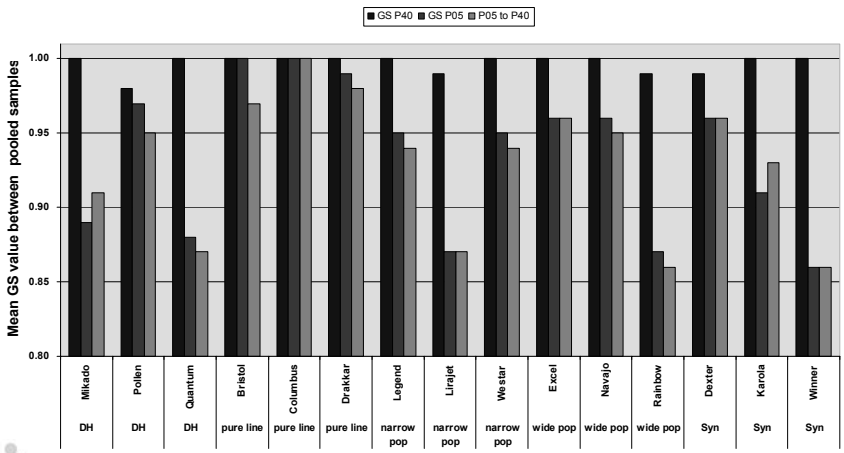
Second Phase Results (1)

b) using pooled samples to increase discrimination power

Average genetic similarities

between bulk-40 samples mutually, bulk-5 samples mutually, and between bulk-5 and bulk-40 samples



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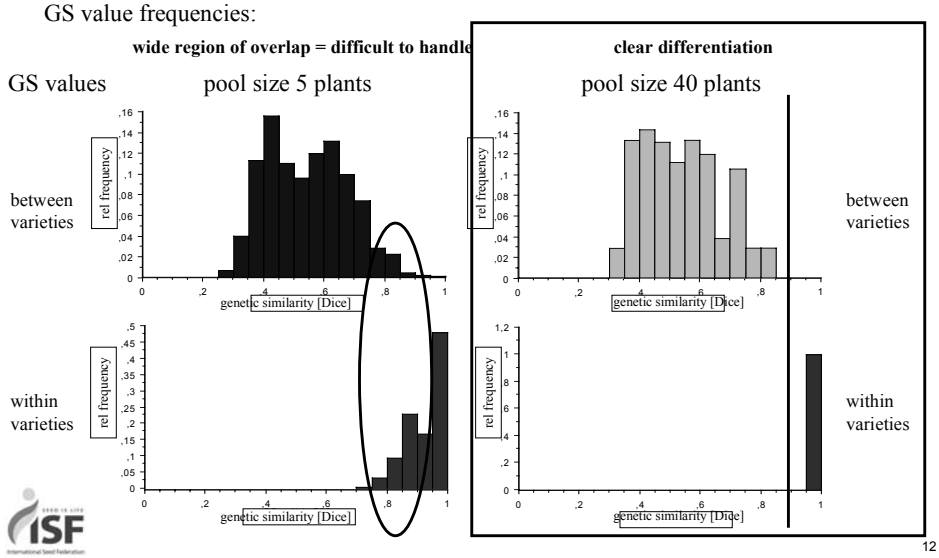
Second Phase Results (2)

b) using pooled samples to increase discrimination power

GS value frequencies:



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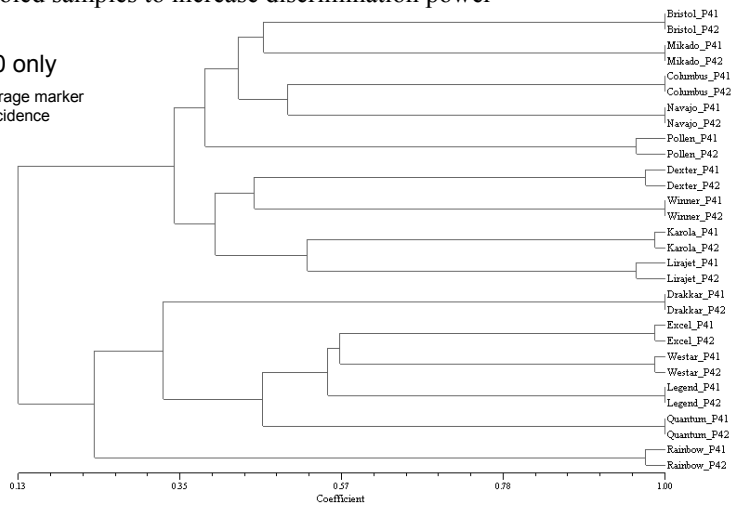
Second Phase Results (3)

b) using pooled samples to increase discrimination power

Bulks of 40 only

Similarity is average marker phenotype coincidence



Note the large distances between the varieties,

-> no discrimination problems between varieties

Conclusions of Second Phase

- Bulks of 40 plants have a very high repeatability
- Bulks of 40 plants lead to a clear separation of all the varieties

Third Phase (1)

- Variation caused by multiplication steps
- Variability of representative sets of commercialized varieties (50 winter and 50 spring varieties)
- Possible threshold based on the upper-tail principle

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Third Phase (2)

ISF-Study 3 – a short overview

Variety sets and markers

c) maintainance breeding

ISF3-001 to 0025	4 winter	5 lots	80 SSR
	1 spring	5 lots	80 SSR

d) larger variety sets

ISF3-W001 to -W050	22 winter	1 x Pool40	80 SSR
	28 winter	1 x Pool36	74 SSR (database)
ISF3-S001 to -S050	37 spring	1 x Pool40	80 SSR
	13 spring	1 x Pool40	74 SSR (database)

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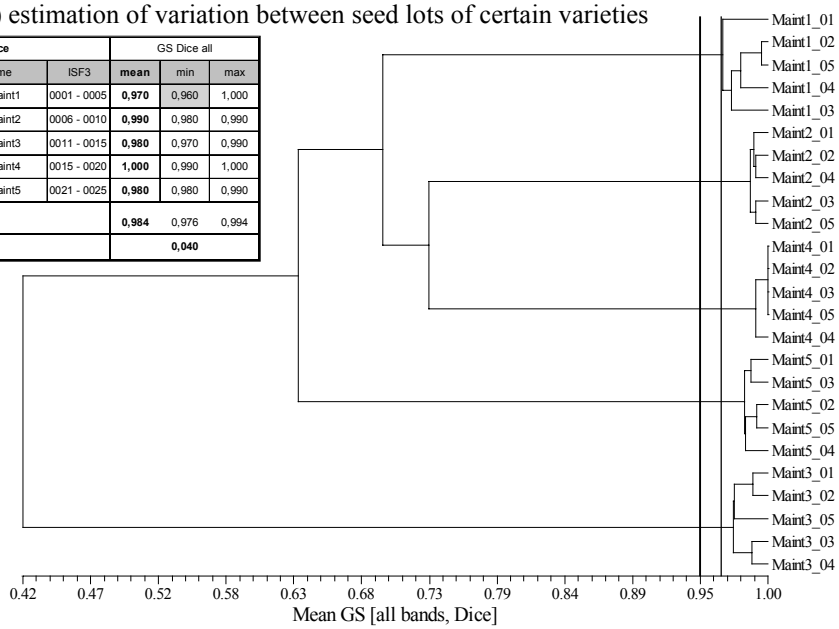
Third Phase Results (1)

c) estimation of variation between seed lots of certain varieties

Dice		GS Dice all		
name	ISF3	mean	min	max
Variety Maint1	0001 - 0005	**0,970**	0,960	1,000
Variety Maint2	0006 - 0010	**0,990**	0,980	0,990
Variety Maint3	0011 - 0015	**0,980**	0,970	0,990
Variety Maint4	0015 - 0020	**1,000**	0,990	1,000
Variety Maint5	0021 - 0025	**0,980**	0,980	0,990
average		**0,984**	0,976	0,994
bias max			**0,040**	



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Third Phase Results (2)

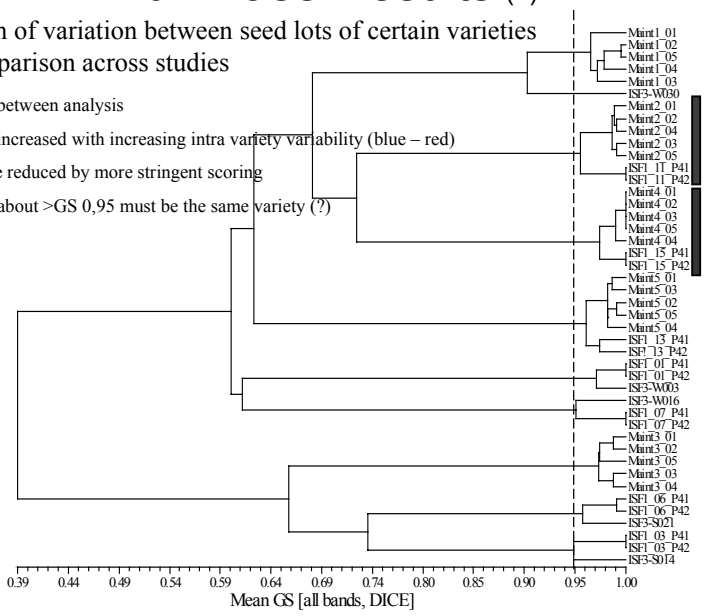
c) estimation of variation between seed lots of certain varieties
- a comparison across studies

-technical error between analysis

-technical error increased with increasing intra variety variability (blue – red)

-probably can be reduced by more stringent scoring

-clearly what is about >GS 0,95 must be the same variety (?)



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Third Phase Results (3)

- According to some assumptions on the varieties under code, it seems that seed lots analyzed during other studies present a higher distance, but in any case higher than 0.95 when using the DICE coefficient with all bands

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Third Phase Results (4)

- For the definition of a possible edv threshold based on the upper-tail approach, we need to have homogeneous sets of genotypes

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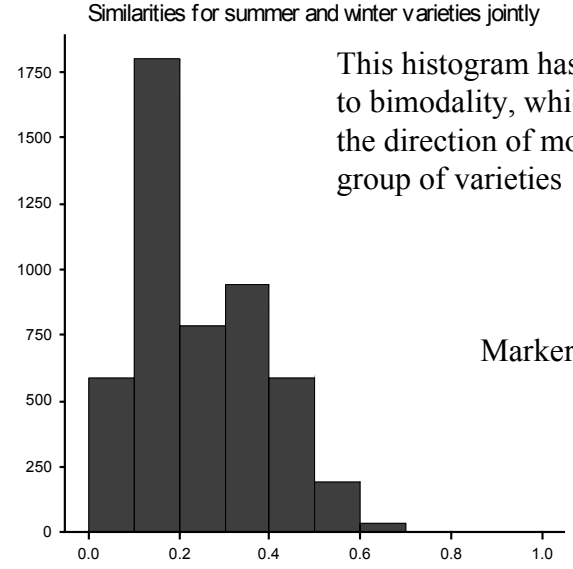
Third Phase Results (5)



This histogram has a tendency to bimodality, which points in the direction of more than one group of varieties

Marker based

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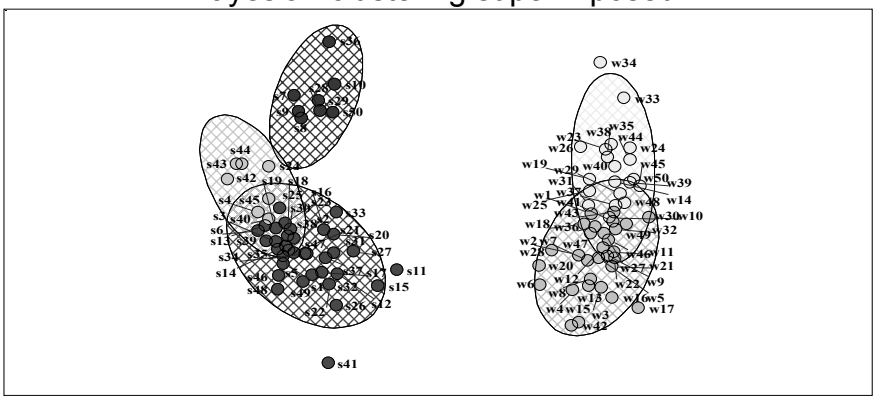
Third Phase Results (6)

MDS on winter + summer varieties with 5 group solution of Bayesian clustering superimposed



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Third Phase Results (7)

- The spring and winter varieties seem to differ in genetic constitution => it is appropriate to address these groups separately for studying the distribution of genetic similarities
- Within the spring and winter varieties there does not seem to exist different genetic structure that would necessitate further splitting => continue the upper-tail investigation for each population separately

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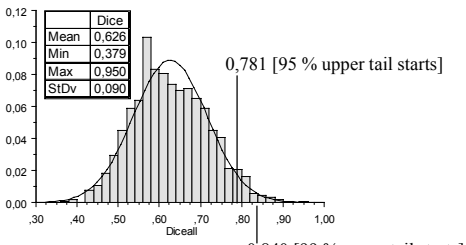
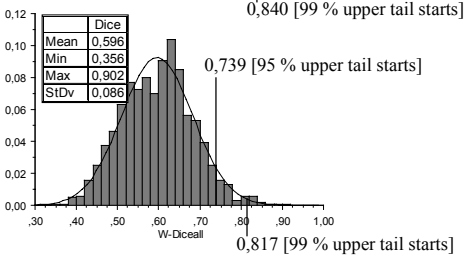
Third Phase Results (8)

Distribution of genetic similarity values calculated using Dice coefficient

spring



winter



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Third Phase Results (9)
Conclusions for thresholds

- On the basis of fitted distributions, an EDV threshold for the summer varieties could be 0.78 (95 percentile), while for winter varieties this could be 0.74 (95 percentile).
- A threshold of 0.76 could be acceptable for both summer and winter varieties

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General Conclusions

- The method used allows:
  - Clear differentiation of the varieties whatever their genetic structure
  - Evaluation of the genetic distances between varieties
  - Similarity between seed lots of the same variety
    - 0.99 or more for pure lines
    - 0.96 or more for synthetics and populations
- The upper-tail approach allows to define possible thresholds for potential essential derivation

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Thank you for your attention