



BMT/11/11 Add.

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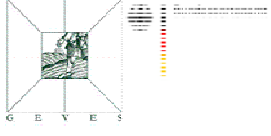

INTERNATIONAL UNION FOR THE PROTECTION OF NEW VARIETIES OF PLANTS
GENEVA

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

Eleventh Session
Madrid, September 16 to 18, 2008


ADDENDUM TO DOCUMENT BMT/11/11
A RESEARCH PROJECT CO-FINANCED BY THE
COMMUNITY PLANT VARIETY OFFICE OF THE EUROPEAN COMMUNITY (CPVO):
“MANAGEMENT OF WINTER OILSEED RAPE REFERENCE COLLECTIONS”

Document prepared by experts from the United Kingdom




EUROPEAN UNION
COMMUNITY PLANT VARIETY OFFICE

Management of Winter Oilseed Rape Reference Collections



A project co-financed by CPVO
Jan 2005- Jan 2008



Collaboration

- NIAB (UK)
- GEVES (France)
- DIAS – now the Plant Directorate (Denmark)
- BSA (Germany)

■ NIAB, GEVES and DIAS conducted molecular analysis – all partners provided phenotypic data

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The Problem....

- **Increasing size of reference collections in EU countries**
- **All varieties of “common knowledge” should be included**
- **A pragmatic approach is needed**
- **Reference collections could be managed by the use of molecular markers**


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Size of reference collections between 2003 and 2006

- **UK ~500 >700 varieties (addition of EU varieties for harmonisation of reference collections)**
- **DK ~490 > 510 varieties**
- **DE ~598 > 696 varieties**
- **FR – Use GAIA**


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 ***DUS Winter oilseed rape plots***






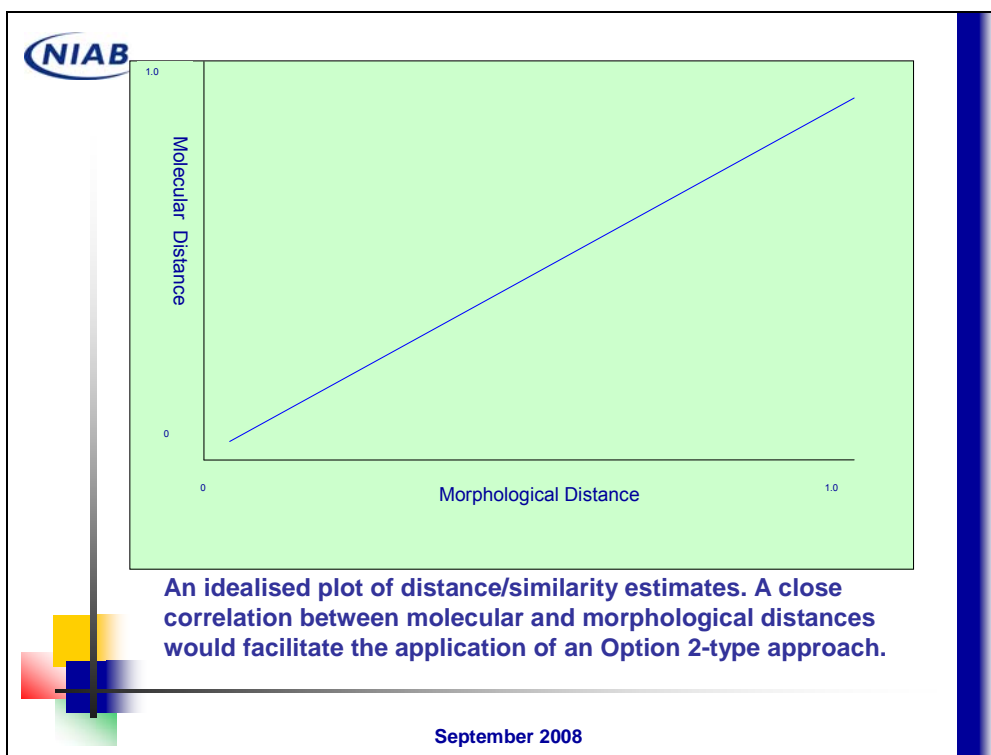
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 ***UPOV Options***

- **Option 1: Molecular characteristics as a predictor of traditional characteristics**
 - (a) Use of molecular characteristics which are directly linked to traditional characteristics (gene specific markers)
 - (b) Use of a set of molecular characteristics which can be used reliably to estimate traditional characteristics; e.g. quantitative trait loci
- **Option 2: Calibration of threshold levels for molecular characteristics against the minimum distance in traditional characteristics**
- **Option 3: Development of a new system**



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- ## *Objectives and approach*
- Evaluate the use of markers to manage the WOSR reference collection
 - Assess an Option 2 approach
 - Maintain the value of variety protection
 - Provide an assured method of pre-selection of varieties to grow via a European database to manage the size of the WOSR reference collection
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Genotyping (1st stage)

- **40 common varieties analysed with 23 publicly available SSR markers in 3 labs using the same seed source**
- **Markers used which were considered to be robust and informative**
- **Different platforms used in this phase (NIAB and DIAS – capillary, GEVES – gel)**

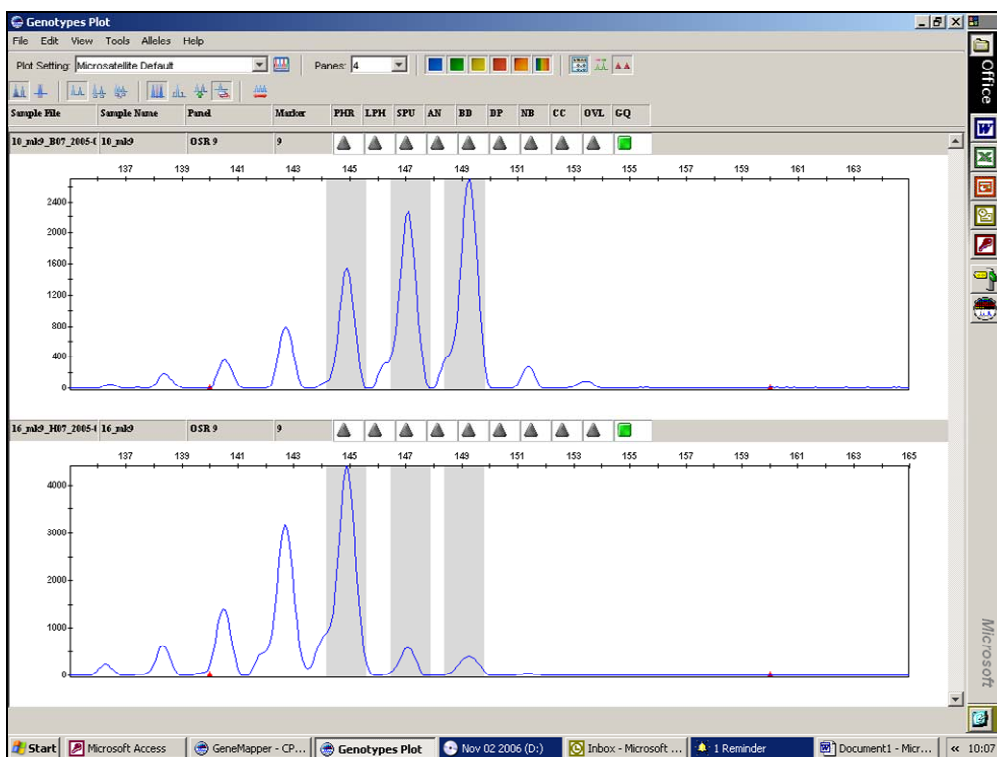
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Genotyping (2nd stage)

- **410 varieties (OP lines) analysed between 3 labs using 23 SSRs**
- **NIAB and GEVES – 190 varieties each, DIAS 70 varieties**
- **NIAB and GEVES – 40 common varieties**
- **All labs - 5 varieties from the original 40**
- **The same platforms used in this phase (all used capillary based)**


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Phenotypic data

- Phenotypic data from 21 CPVO characteristics used: UPOV notes and year means (2003, 2004 and 2005)
- Data from all partners collated for the same 410 varieties genotyped using the selected SSRs



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Phenotypic data

- MODAL note was obtained where possible for the notes data
- Where too little data the MEDIAN value was used
- A REML analysis was run to generate the predicted variety mean value
- Euclidean and City Block (Gower's method) were used to compute phenotypic distances from mixed data types

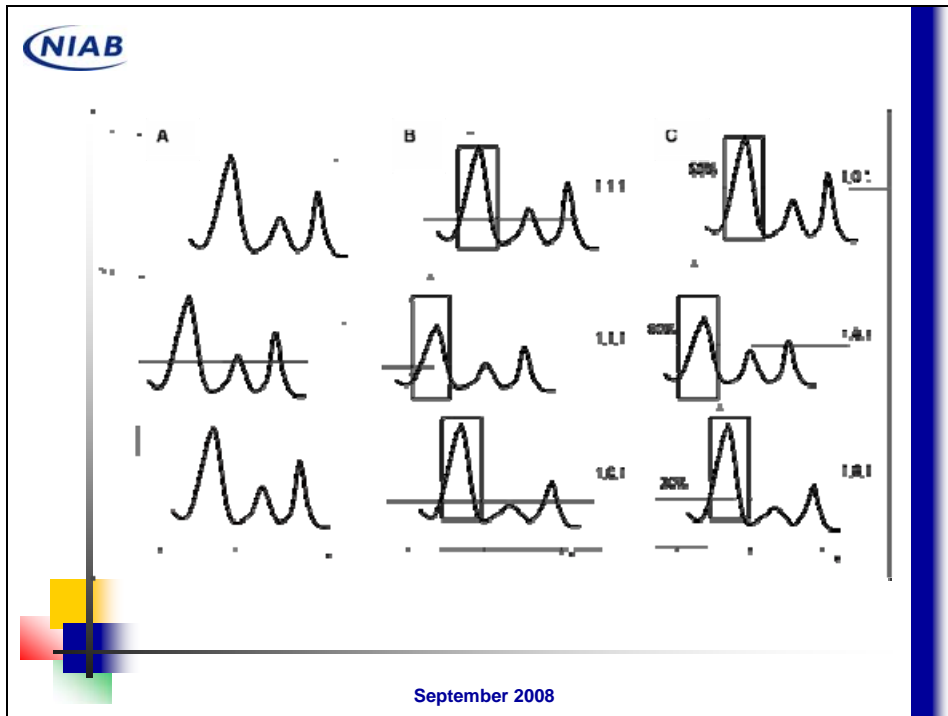
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Molecular data

- Relative responses for each allele differed between labs
- Thresholding of the data was carried out to standardise results between labs
- Five different methods for establishing genetic distance were tested (DICE, Jaccard, Ochiai, Sokal & Sneath and Simple Matching)

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Relative thresholding

- Relative thresholding produced 3 data sets
- 90% concordance with 18 markers
- 95% concordance with 11 markers
- 90% concordance with 14 markers

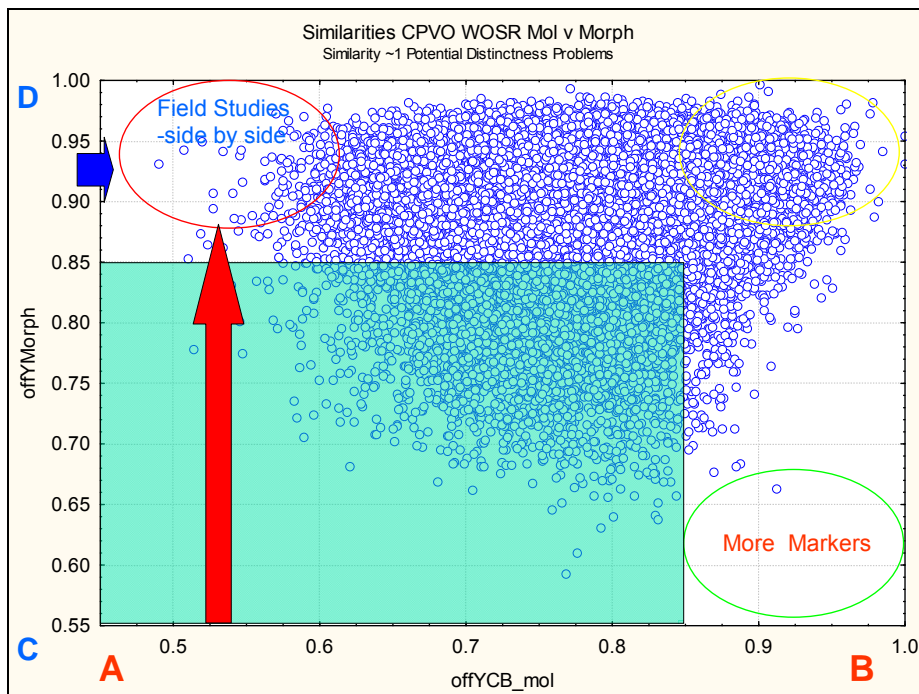
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NIAB

Data analysis

- Genetic and phenotypic distances calculated and compared
- Lists of interesting variety pairs compiled for each country
- Variety pairs selected which were morphologically close whilst being very different using markers
- Selected variety pairs from each country sown in field trials and duplicated in all countries (August/September 2006)

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Results – molecular data

- Good quality data produced from 335 varieties and 18 markers.
- Genetic distance indices were highly correlated between methods used.

	Dice	Jaccard	Ochiai	Sokal & Sneath	Simple Matching
Dice	1				
Jaccard	0.996	1			
Ochiai	0.999	0.996	1		
Sokal & Sneath	0.990	0.987	0.990	1	
Simple Matching	0.980	0.994	0.980	0.973	1

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Results molecular data

- The verified molecular data were demonstrated to be “fit for purpose”
- Number of markers used affects the absolute values of the distances but not the correlation between methods
- The choice of method used is not critical

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Phenotypic and molecular distances

- There was no correlation between any of the morphological and molecular estimates of similarity (<0.1)
- Variety pair similarity showed the morphological and molecular similarities only agreed on 3 of a possible 55,000 pairs

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Conclusions

- There is no relationship between morphology and genetic similarity based on these data
- Morphological similarity cannot be predicted directly from genetic similarity
- It is not possible to apply UPOV "Option 2" as originally conceived

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Conclusions

**Although Option 2 requires
“Calibration of threshold levels for
molecular characteristics against the
minimum distance in traditional
characteristics “.....**

**Results from the project indicate that it
may be possible to define those
thresholds, given further study**

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Conclusions

- **The use of molecular markers in combination with phenotypic characteristics could be a way to reduce the number of varieties grown in the field**
- **One such approach investigated in the project is to use markers in combination with GAIA**

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Further work needed

- **The use of more and better SSRs (dispersed throughout the genome)**
- **The use of functional SSRs and/or SNPs**
- **An assessment of robust characteristics that can easily be combined**




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Acknowledgements

- **CPVO for co-funding the whole project**
- **DEFRA for co-funding in UK.**


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NIAB (GB), GEVES (F), DIAS (DK), BSA (D)



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
A RESEARCH PROJECT CO-FINANCED BY CPVO:
"MANAGEMENT OF WINTER OILSEED RAPE REFERENCE COLLECTIONS"

Coordinator: NIAB, United Kingdom 




Partners:

Bundessortenamt (BSA), Germany 

Danish Institute of Agricultural Sciences (DIAS), Denmark
now the Plant Directorate 

Groupe d'Etude et de contrôle des Variétés Et des Semences (GEVES), France 



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**Use of molecular distances in combination with GAIA
for the management of WOSR reference collection**

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GAIA

GAIA is a software developed by GEVES to pre-select the reference varieties to compare in the field to the new varieties for DUS testing.



For WOSR, it is based on the accumulation of differences between varieties observed on qualitative and quantitative **morphological characteristics and isoenzyme data**.

Those differences are given **weights** (previously defined by crop experts): a difference between more reliable characteristics being given a greater weight than a difference between less reliable ones.


→ **an index is calculated for each pair of varieties (GAIA index)**

For WOSR, all pairs of varieties with a **Gaia index ≥ 6** are considered “**super distinct**” and are not put in the field for comparison to the new varieties.

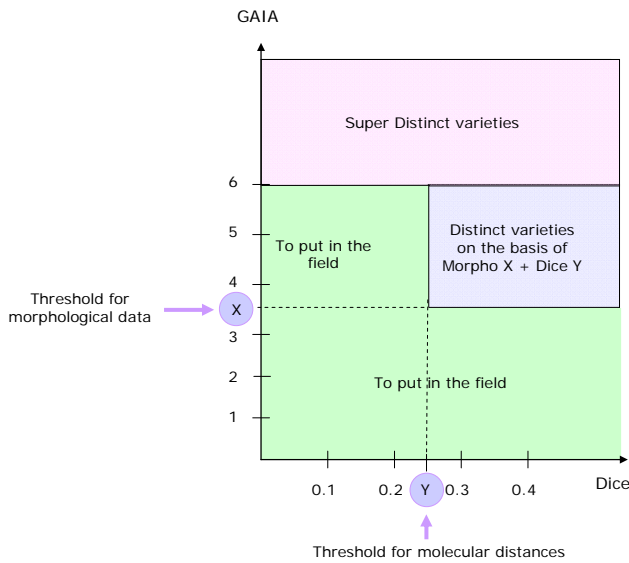
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GEVES proposal to combine morphological and molecular distances to select variety pairs to be studied in the field:



The graph plots GAIA index (y-axis, 1 to 6) against Dice distance (x-axis, 0.1 to 0.4). A horizontal dashed line at GAIA = X (labeled 'Threshold for morphological data') and a vertical dashed line at Dice = Y (labeled 'Threshold for molecular distances') intersect. The area above GAIA = 6 is pink and labeled 'Super Distinct varieties'. The area below GAIA = 6 and to the left of Dice = Y is green and labeled 'To put in the field'. The area to the right of Dice = Y is light blue and labeled 'Distinct varieties on the basis of Morpho X + Dice Y'.

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Morphological distances

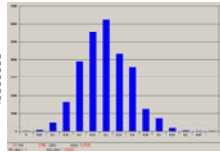
- Common data base of the programme (335 varieties, 55 945 pairs)
- Distances calculated by GAIA software (GEVES) : usual threshold for distinction = 6
- GAIA distance thresholds tested : 3, 4, 5

Molecular distances

- Data from molecular set 1 (18 markers)
- Distances calculated by Darwin software (CIRAD)
- Dice distance thresholds tested : **0.35; 0.3; 0.25; 0.2 and 0.15**

Methods compared

- only morphological characteristics
- morphological and electrophoresis characteristics
- morphological and molecular characteristics (Dice distances)



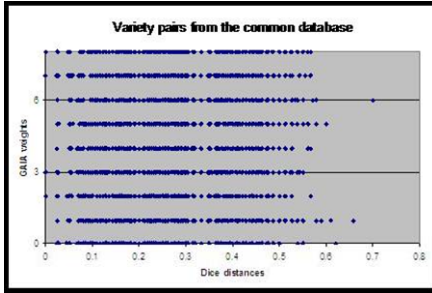
Mean around 0.3
Min-max : 0 – 0.7

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Distribution of GAIA weights according to the Dice distance

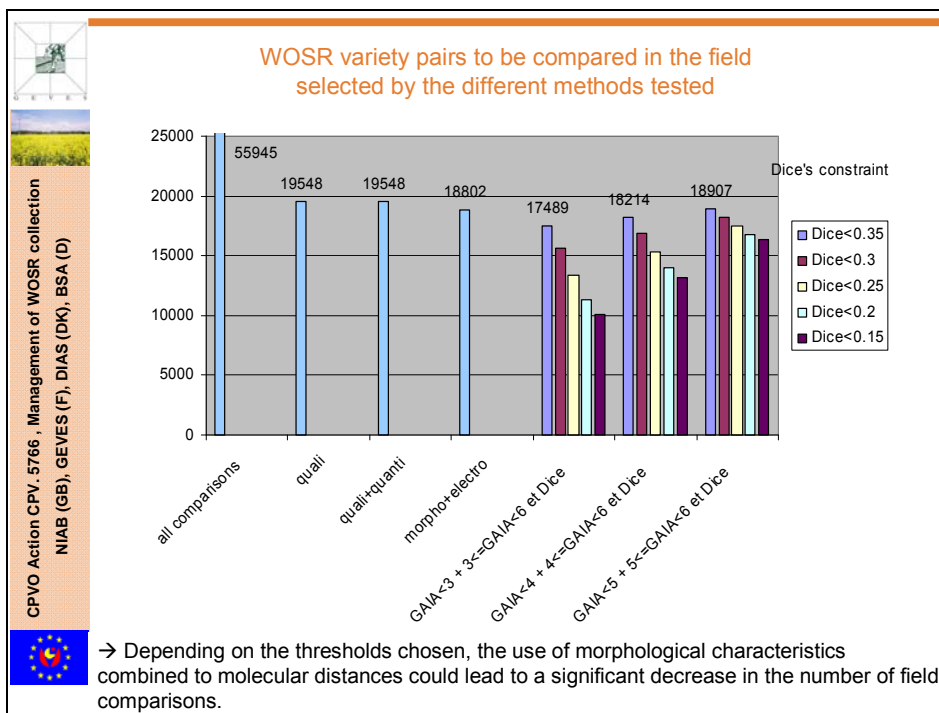
Focus on the Dice distribution for the variety pairs with GAIA weight < 6



→ Based on the varieties of this database and on the molecular markers used, no correlation can be observed between Dice distances and GAIA weights.

These observations support the idea that molecular distances can not replace phenotypic characteristics but should be combined to them, with appropriate thresholds, to select the variety pairs that need to be compared side by side in the field.

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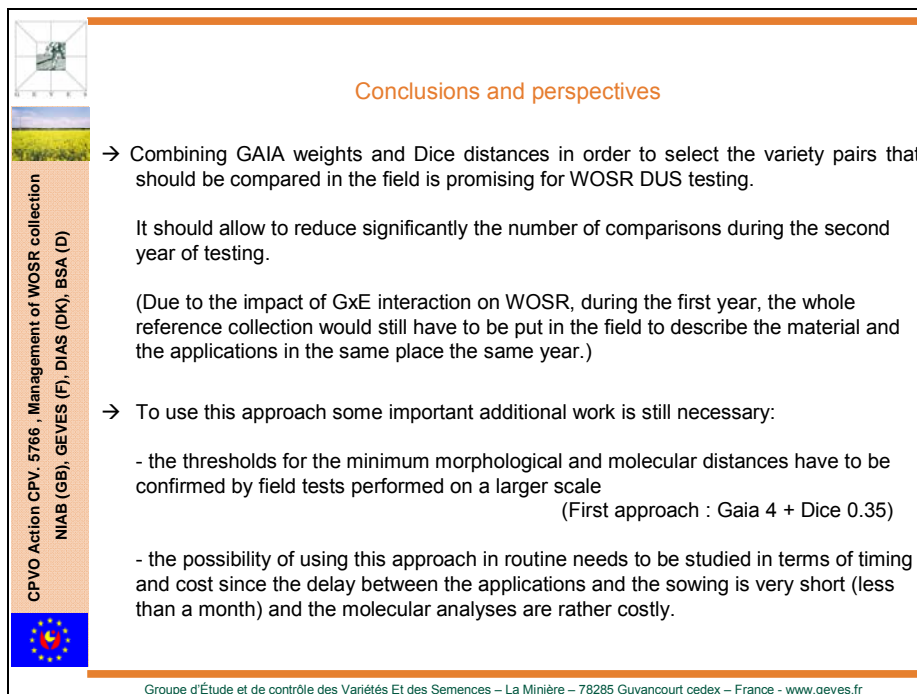
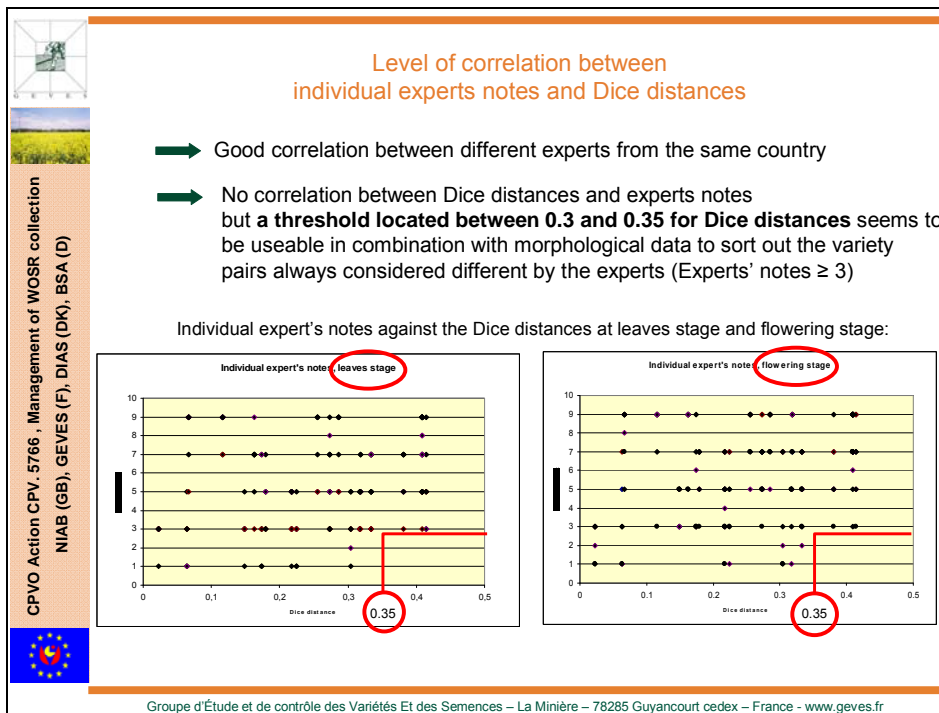
How to define the appropriate molecular threshold ?

→ We decided to use “the expert’s appreciation of the degree of similarities/differences” between varieties and to compare it to molecular distances :

- **Material** : 26 variety pairs with various phenotypic and molecular distances
- **Field design** : pairs of varieties grown side by side (1 plot = 2 rows of 15 plants)
- **Visual assessment of the global phenotype by WOSR crop experts**
2 observation stages : leaves / flowering
1 to 4 experts depending on countries
- **Scale of similarity:**
 1. the two varieties are similar or very close
 3. the two varieties are distinct but close
 5. the comparison was useful, but the varieties are clearly distinct
 7. the comparison should have been avoided because the varieties are very different
 9. the comparison should have been avoided because the varieties are totally different

The experts notes were then compared to the corresponding Dice distances.

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