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UPOV

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

COMBINING MOLECULAR DISTANCES TO MORPHOLOGICAL CHARACTERISTICS FOR THE MANAGEMENT OF FIELD COMPARISONS IN SPRING BARLEY

Document prepared by experts from France

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Eleventh Session, Madrid, September 16 to 18, 2008

Combining molecular distances to morphological characteristics for the management of field comparisons in spring barley.

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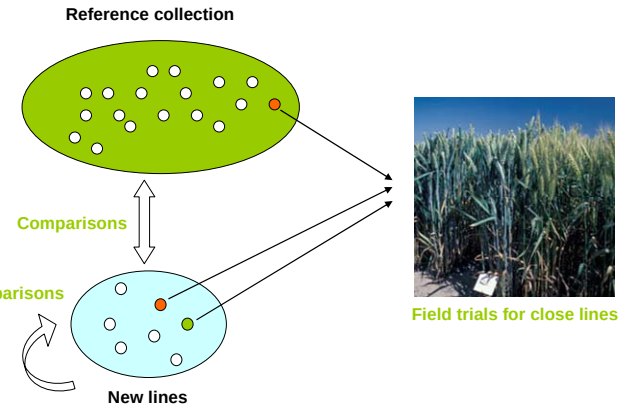
GEVES - France

DISTINCTNESS PROCEDURE



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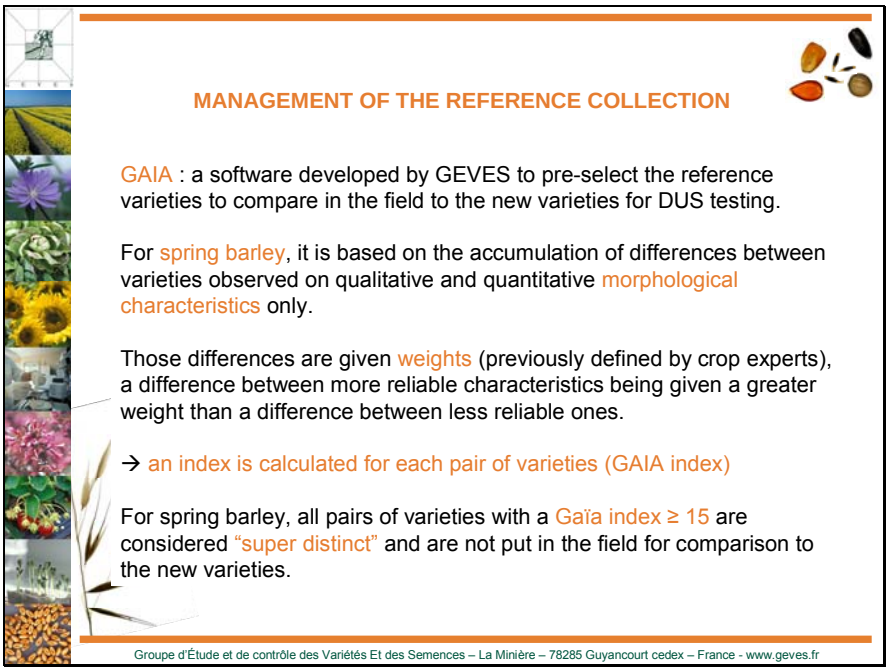
MANAGEMENT OF THE REFERENCE COLLECTION
GAIA : a software developed by GEVES to pre-select the reference varieties to compare in the field to the new varieties for DUS testing.
For spring barley, it is based on the accumulation of differences between varieties observed on qualitative and quantitative morphological characteristics only.
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 spring barley, all pairs of varieties with a Gaïa index ≥ 15 are considered "super distinct" and are not put in the field for comparison to the new varieties.
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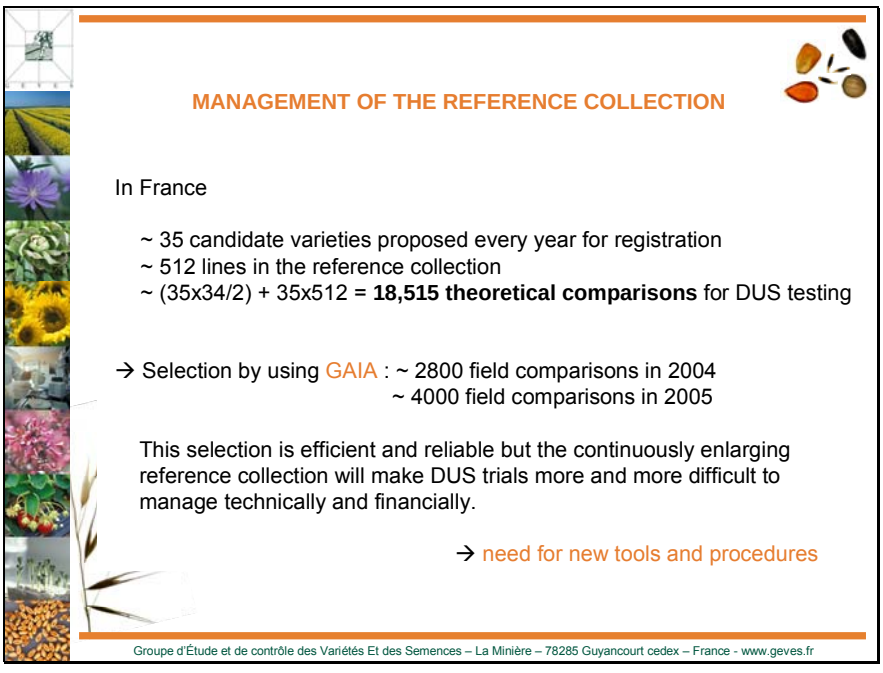
MANAGEMENT OF THE REFERENCE COLLECTION
In France
~ 35 candidate varieties proposed every year for registration
~ 512 lines in the reference collection
~ (35x34/2) + 35x512 = 18,515 theoretical comparisons for DUS testing
→ Selection by using GAIA : ~ 2800 field comparisons in 2004
~ 4000 field comparisons in 2005
This selection is efficient and reliable but the continuously enlarging reference collection will make DUS trials more and more difficult to manage technically and financially.
→ need for new tools and procedures
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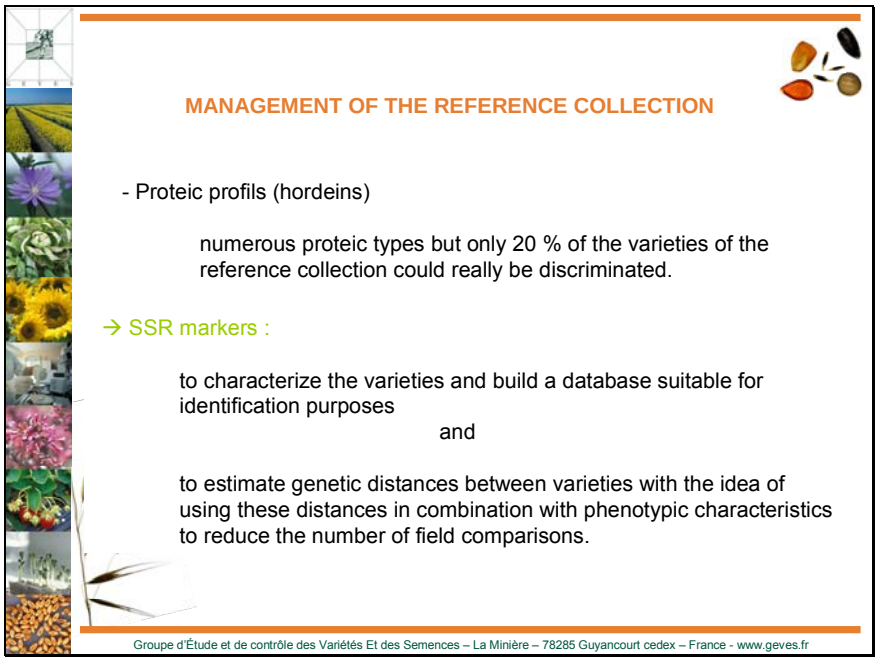
MANAGEMENT OF THE REFERENCE COLLECTION
- Proteic profils (hordeins)
numerous proteic types but only 20 % of the varieties of the reference collection could really be discriminated.
→ SSR markers :
to characterize the varieties and build a database suitable for identification purposes
and
to estimate genetic distances between varieties with the idea of using these distances in combination with phenotypic characteristics to reduce the number of field comparisons.
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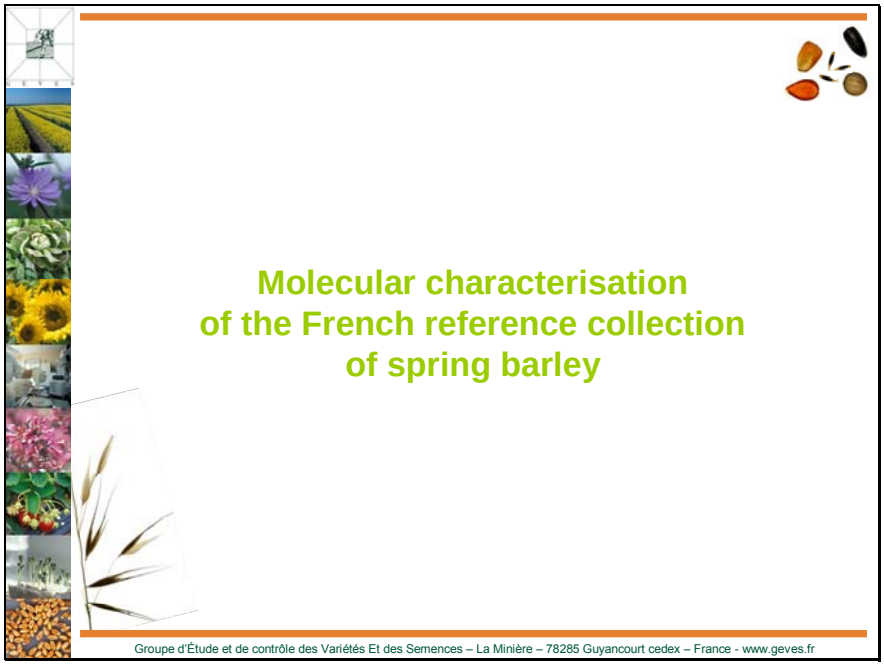
Molecular characterisation
of the French reference collection
of spring barley
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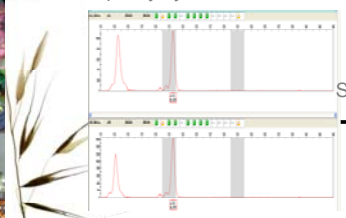
Molecular characterisation of the French reference collection of spring barley

Test on a subset of 12 varieties of 36 SSR markers from Ramsay et al. (2000) and 48 published by Macaulay et al. (2001)

→ 30 SSR markers with
- a good coverage of the genome
- a high level of polymorphism
- an easy scoring

Description of 512 varieties from the French reference collection on bulked samples of 20 seeds.

ABI capillary system



Scoring →

Data base

	SSR	phi 109275						phi 015					
cultivars	N°var	158	155	152	149	143	Nu1	122	116	110	107	104	101
105721	3	.	.	.	.	.	.	.	.	.	.	.	.
138082	4	1	0	0	0	0	0	1	0	0	0	0	0
157626	5	1	0	0	0	0	0	0	1	0	0	0	0
143011	6	0	0	0	1	0	0	0	1	0	0	0	0

Molecular characterisation of the French reference collection of spring barley

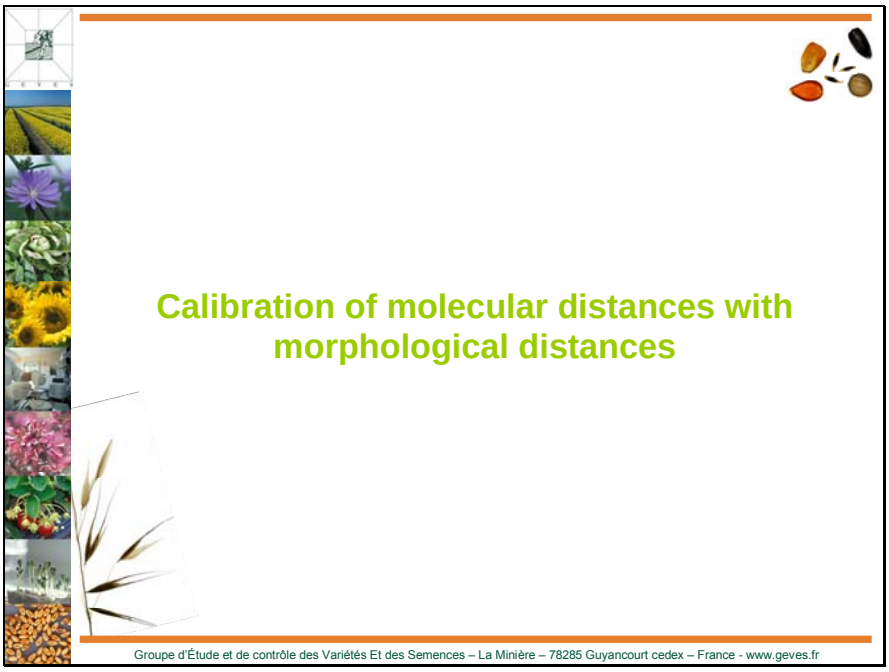
The analysis generated 234 alleles
7.83 per SSR (min 6 ; max 19)

The average PIC was of 0.58

These results enabled to discriminate all varieties except 12 pairs.

58 % of varieties displayed heterogeneity on at least 1 locus.
Only 13.8% were heterogeneous on more than 2 loci.

The average level of heterogeneity revealed per locus was 2.2% which is very close to what is observed on maize and can be considered as low.


Calibration of molecular distances with morphological distances
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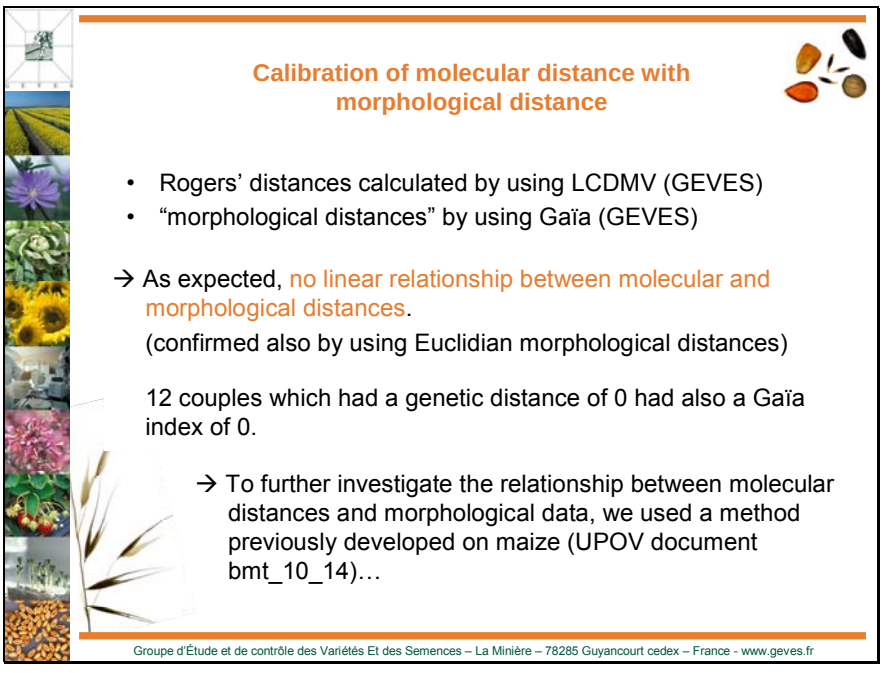
Calibration of molecular distance with morphological distance
• Rogers' distances calculated by using LCDMV (GEVES)
• "morphological distances" by using Gaïa (GEVES)
→ As expected, no linear relationship between molecular and morphological distances.
(confirmed also by using Euclidian morphological distances)
12 couples which had a genetic distance of 0 had also a Gaïa index of 0.
→ To further investigate the relationship between molecular distances and morphological data, we used a method previously developed on maize (UPOV document bmt_10_14)…
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Calibration of molecular distance with morphological distance

EXPERTS' APPRECIATION OF THE DEGREE OF SIMILARITY/DIFFERENCE BETWEEN VARIETIES

- **Material** : 152 pairs of varieties with various GAIA indices and molecular distances

- **Field design** : pairs of varieties grown side by side in two locations (La Minière & Le Magneraud)
  (1 plot = 2 rows of 15 plants)

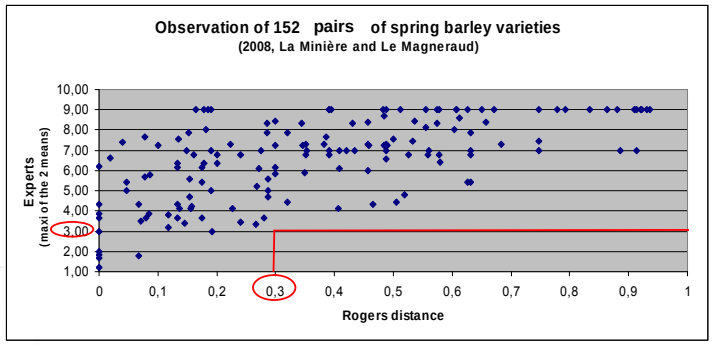
- **Visual assessment** by 11 spring barley crop experts who individually scored the pairs of varieties for their distinctness on a scale of similarity ranging from 1 to 9

- **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

For each variety pair, the scores from the 11 experts were averaged for each location. The maximum value of the 2 means was considered as the experts' note on to each pair.

The experts' notes were then compared with the Rogers distances.

Calibration of molecular distance with morphological distance



In our experiment, all the variety pairs with Rogers distances > 0.3 had experts' notes ≥ to 3 (meaning "distinct varieties").

→ A Rogers distance of 0.3 was thus considered as a potential molecular threshold for the selection of field comparisons in combination with morphological data.

Proposal of an approach combining molecular and morphological distances for the management of reference collection

Proposal of an approach combining molecular and morphological distances

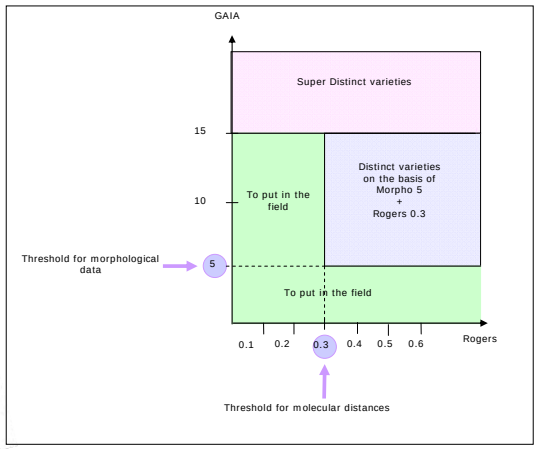
In addition to the identification of a threshold for molecular distance, our approach relies on the determination of a minimum requirement for morphological differences, meaning a minimum GAIA index below which all variety pairs have to be studied in the field.

This minimum requirement for morphological differences have to be defined by crop experts.

For spring barley, French experts are open to consider a GAIA weight between 5 and 10. (This still has to be discussed with other experts).

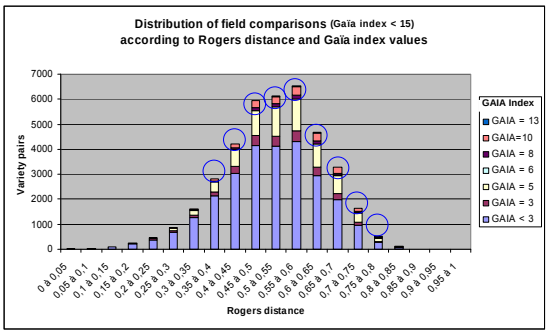
Unlike some other species like maize, spring barley shows a rater low variability based on the UPOV characters. The minimum GAIA weight accepted by crop experts will have to take it into account in order to reduce the risk of discarding varieties that would not be distinct from the new lines.

Proposal of an approach combining molecular and morphological distances

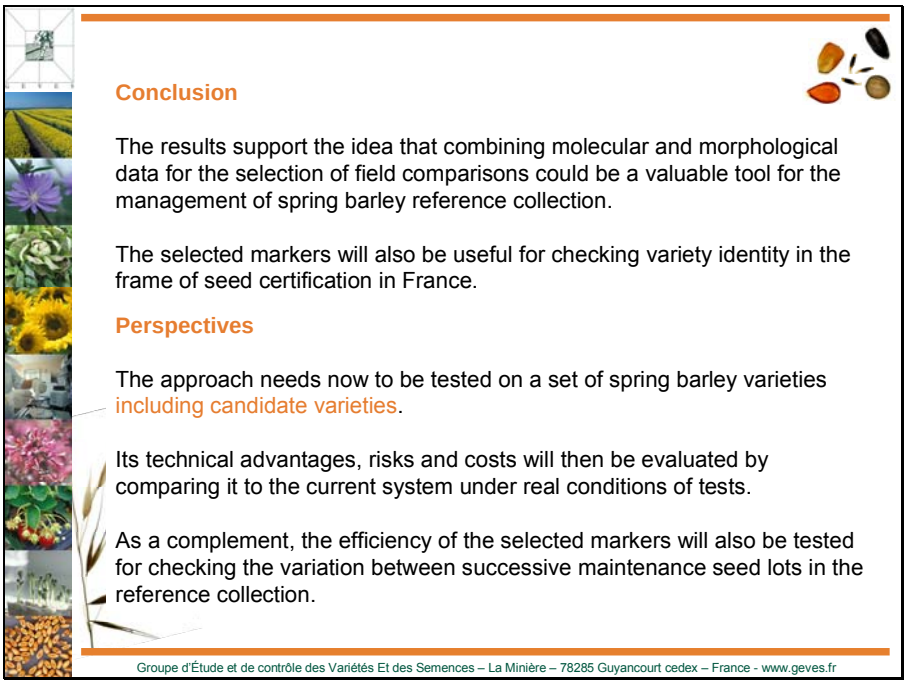


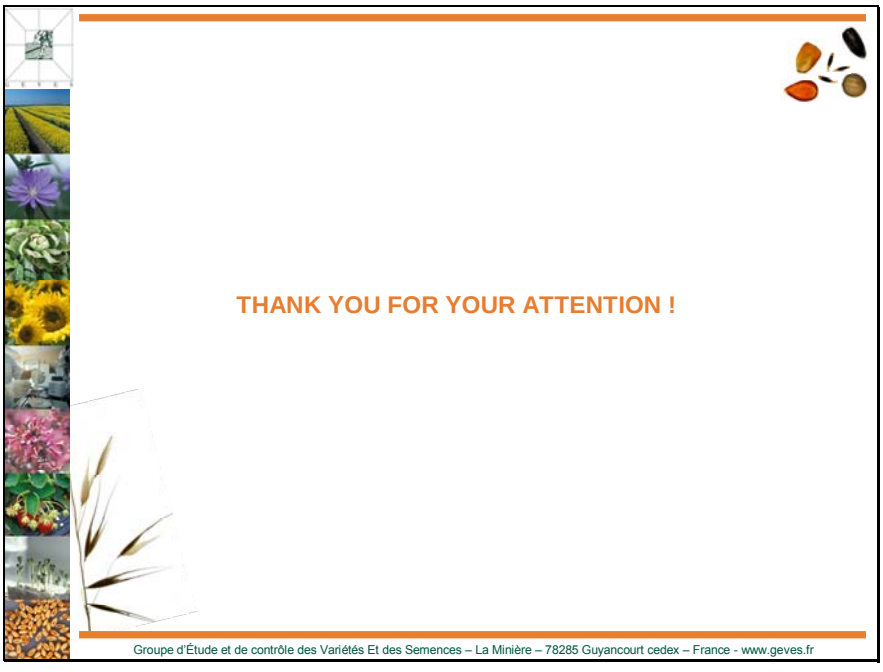
Preliminary results

By applying the "combined approach" with Gaia index > 5 and Rogers distance > 0.3, we think that a significant number of field comparisons could be avoided (blue circles) :



→ The exact number of the field comparisons that could be avoided still has to be determined precisely by including candidate varieties in the analysis.





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