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Geneva

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

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ADDENDUM TO DOCUMENT BMT/14/17

IMPROVING EFFICIENCY OF DUS TESTING OF PERENNIAL RYEGRASS BY COMBINING MORPHOLOGICAL AND MOLECULAR VARIETY DISTANCES

Document prepared by experts from Expert from the Netherlands

The Annex to this document contains a copy of a presentation “The use of molecular markers (SNP) for maize DUS testing” made at the fourteenth session of the Working Group on Biochemical and Molecular Techniques and DNA-Profiling in particular (BMT).

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[Annex follows]

Improving the efficiency in DUS testing of grasses

Henk Bonthuis & Hedwich Teunissen,
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Lolium perenne (perennial ryegrass)

- Genetically diverse due to:
  - Obligate outcrossing species
  - Synthetic varieties: varieties are genetically heterogenic populations
  - created by polycross of selected individual clones
- Morphologically diverse due to:
  - Genotype environment interactions

DUS procedure in L. perenne

- UPOV protocol TG/4/8
- DUS trials take 2 or 3 years
- DUS testing is based on measurements and statistics
- Most morphological characteristics are quantitative traits
- A large collection of reference varieties is needed to judge candidates in the trial

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Limitations and risks

- DUS testing is based on measurements and statistics
  - The measurements are complicated, labour intensive, time consuming and very expensive
- Most morphological characteristics are quantitative traits
  - These traits are consistent but variable due to environmental factors
  - As a result the distinctness between varieties is often not significant
  - In contrast, VCU research shows mostly clear differences in yield, resistances etc.
- A large collection of reference varieties is needed to judge candidates in the trial
  - Expensive

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Objectives

How to improve the efficiency of DUS testing in L. perenne?

Limiting the number of reference varieties by:

- ✓ Combining morphological distances and molecular distances (UPOV/INF/18/1)

Making morphological differences more predictable by:

- ✓ Creating molecular databases for DUS screening by EOs and breeders on forehand

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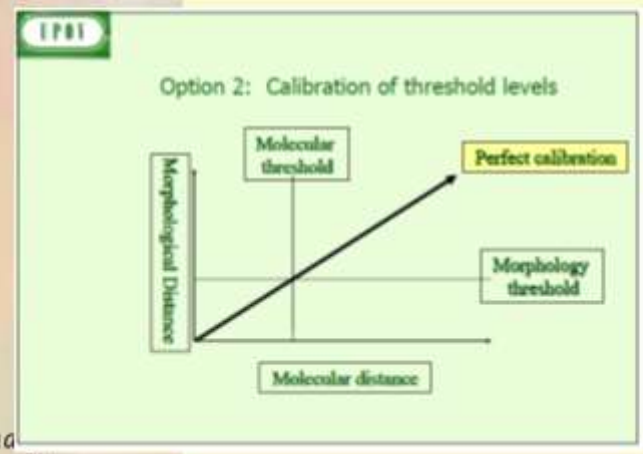
Correlation?

Perfect correlation between molecular distance and morphological distance usually does not exist.



(SNP) Markers up to now are unrelated to morphology !

Minimizing the likelyhood of incorrect decisions

- Despite the fact that molecular markers and morphological characteristiscs are unrelated, they can be combined to determine the threshold for incorrect decisions on reference varieties to be excluded from the field trial on forehand.
- Pilot study on probability analyses

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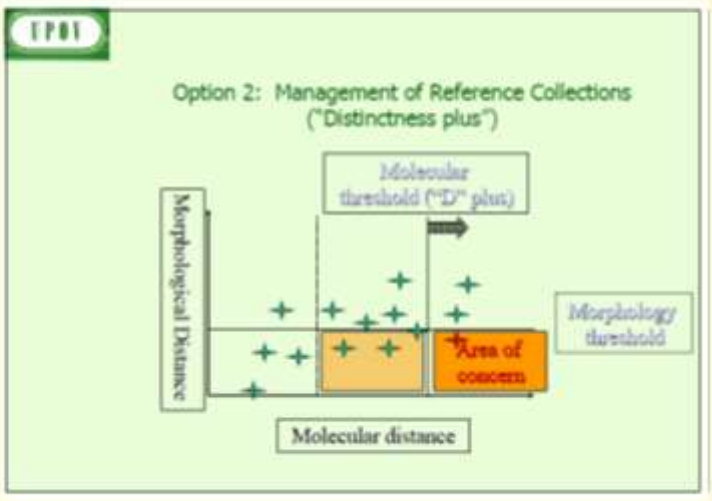
UPOV model 2 approach:

Area of Concern = the probability of incorrect decisions on excluding reference varieties from the field trial



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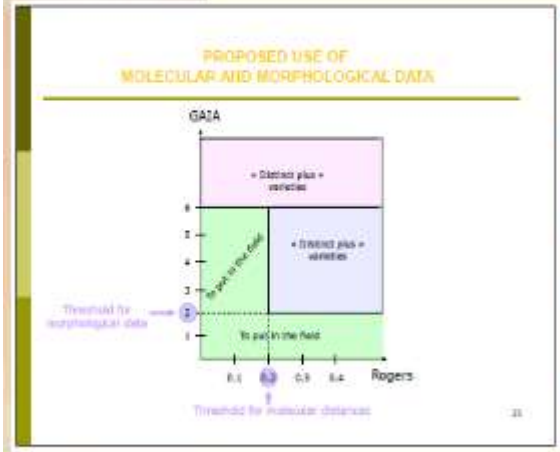
UPOV Model 2 in Maize (France):



- GAIA distance = similarity index for morphological differences
- Rogers distance = molecular diff.
- Pink area = distinct plus = varieties which are distinct "for sure"
- Green area = reference varieties to be included in the field trial
- Purple area = varieties which can be excluded from the field trial.
- Project objective: maximize/optimize the purple area.

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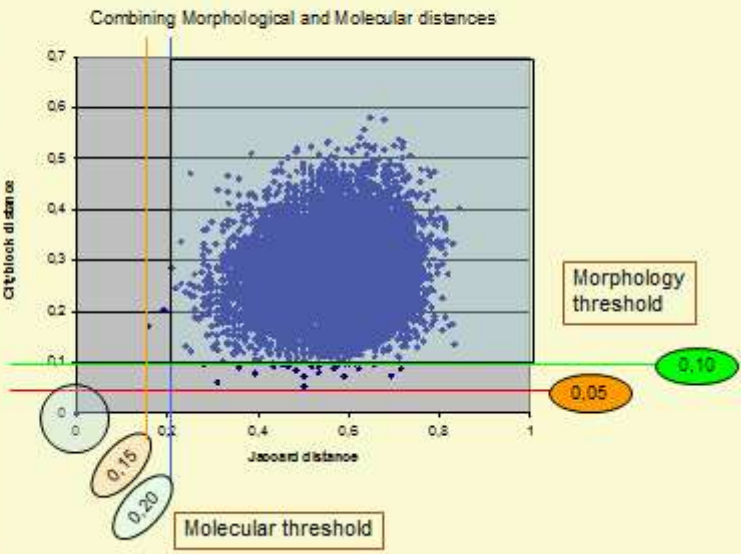
UPOV Model 2 in potato (NL)



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Pilot experiment set-up

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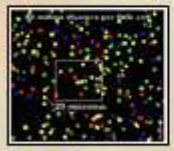
- Phenotypical data of 20 varieties
  - Standard UPOV characteristics determined – TG/4/8
  - measurements of 60 individual plants per variety over 3 years (2010 – 2012). Complete datasets for all selected varieties.
  - Selection includes closely related and non-related varieties (maximum expected diversity)
- Molecular analysis based on SNP detection using a genotyping-by-sequencing (GBS) approach
- 20x19/2 = 190 pairwise combinations

Genotyping-by-Sequencing



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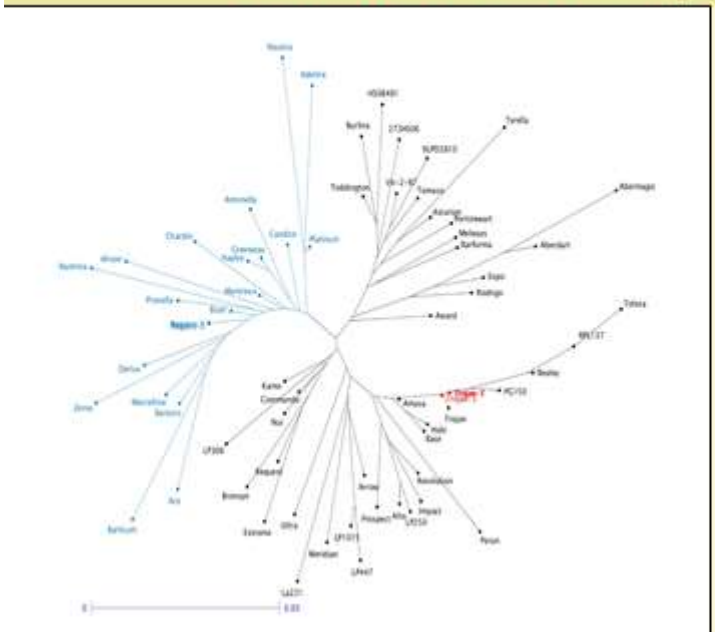
20 varieties of amenity grasses were genotyped by AgriBio lab (Centre for AgroBioscience, Bundoora, Victoria, Australia)

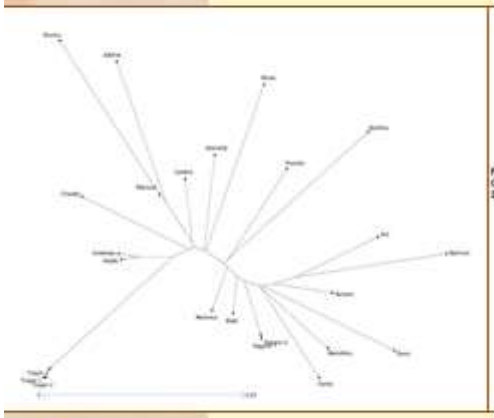
- Each variety 2,5 g seed (1000 seeds = reasonable representation of the variety).
- DNA extraction (DNeasy Plant kit from Qiagen)
- (targeted?) Amplification step (in total 295 SNP-markers were used)
- Ligation using bar-coded synthetic DNA adapters
- Sequencing with the Illumina MiSeq
- Genetic relationships defined by Nei's genetic distance based on allele-frequencies

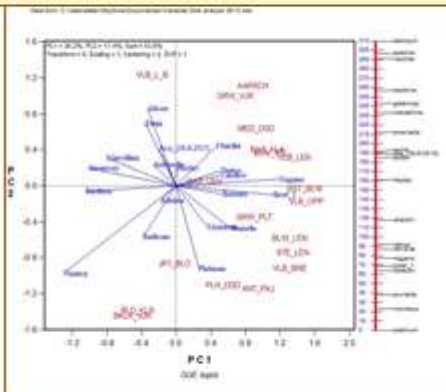
Genetic relationships



Tested cultivars (in blue) and control samples (in red) integrated into the perennial ryegrass catalogue of AgriBio Lab

Molecular distances unrelated to morphology (as expected)





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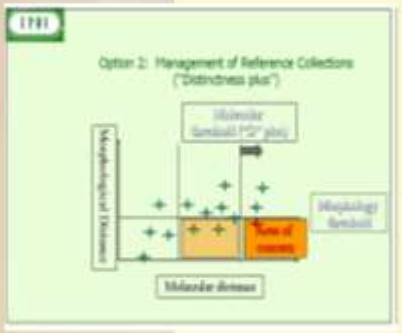
Next step

determine the molecular threshold



- Further Data Analysis
- (Biometris Wageningen UR)
- Calculation of similarity indices and thresholds
- Minimizing the Area of Concern - for a safe decision on reference varieties which should be tested in the field

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Future perspective

- If results are promising:
  - Genotyping of complete collections (or subgroups)
  - Further analysis of similarity indices (on morphology and SNP-information)
  - Practical implementation
- Create consortium of
  - Testing stations (EU, AUS, NZ)
  - DNA-labs with comparable expertise (on GBS)
  - Breeding companies
- Funds available ?
  - Co-financing of European Plant Variety Office (CPVO) ?

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[End of Annex and of document]