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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

THE USE OF MOLECULAR TECHNIQUES WHEN INFRINGEMENT OF PBR OR ESSENTIALLY DERIVATION IS SUSPECTED

Document prepared by an expert from the Netherlands

The use of molecular techniques when infringement of PBR or essentially derivation is suspected

Naktuinbouw
Hedwich Teunissen

Variety Tracer

- The 'Sherlock Holmes' concept in infringement matters

- To answer questions about:
  - Identity of plant material
  - Suspicion of repeated cropping
  - Suspicion of infringement of Plant Breeders' Rights
  - Essentially Derived Varieties

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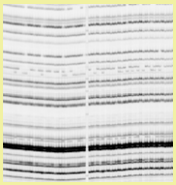
Variety Tracer

Five steps

1. Background information and problem analysis
2. Sampling
3. Morphological assessment
4. DNA analysis
5. Reporting conclusions



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Examples

- **Tilia (lime tree)**

- **Freesia (court case in the Netherlands)**

Tilia

- **Background information and problem analysis**
  - Expectation/demands = batch of authentic Tilia platyphyllos 'orebro', size 14/16 cm, origin Denmark)
  - Suspicion:
    - not from one origin but from several sources
    - batch is mixture of different varieties

Tilia: Sampling and morphological assessment 1

- Observation and description of 280 trees by two independent Naktuinbouw tree specialists
- Observations confirmed suspicion of different origins:
  - Different cut-back methods resulting in variable crown structures and crown shaps
  - Some trees had plenty of leaves, other part did not have leaves left (February)
  - Different ages
- More than one variety?

Tilia: Sampling and morphological assessment 2

- From 280 trees, 12 trees randomly sampled for DNA analysis
- All sampled trees were photographed and color coded
- Sampled branches were grafted on rootstock and grown in greenhouse in NL

Tilia

- **DNA analysis**
  - DNA extraction from 12 test samples Denmark
  - DNA extraction from 12 reference samples of known identity from reference gardens 'vermeerderingstuinen Nederland' Zeewolde, NL
  - Genotyping with AFLP using six primer combinations resulted in 254 markers (presence/absence)

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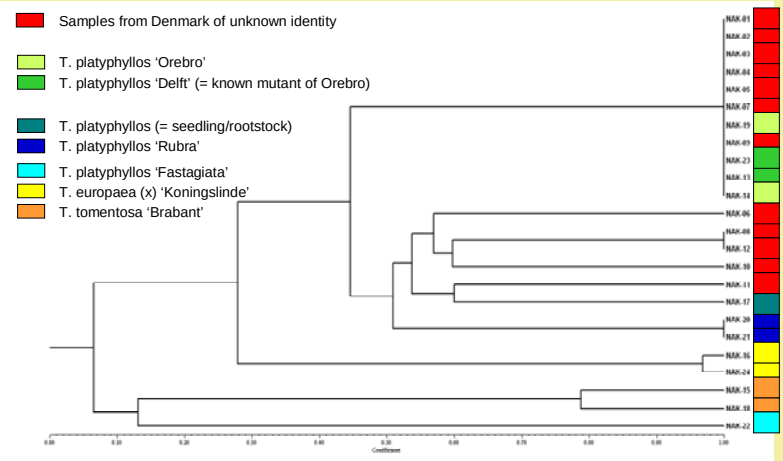
Tilia Clustering analysis – Jaccard



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Tilia conclusions

- 7 of 12 Danish trees comply with agreed conditions (T. platyphyllos 'orebro')
- 5 of 12 Danish trees show divergent fingerprints (T. platyphyllos *but not* 'orebro') and a most likely seedlings

Freesia

- Background information and problem analysis
  - Both variety A (1991) and variety B (2007) have Plant Breeders' Rights
  - Variety descriptions very similar
  - Suspicion of owner variety A: variety B is identical to or essentially derived from variety A

Freesia – Material and experimental set-up

Variety A: Sample 1-3.
Material from different origins

Variety B: Sample 4-7.
Material sequestrated by bailiff at suspected company
Material from different origins

Reference varieties: Sample 8-32.
Material from the DUS trial of 2008 in Wageningen

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Freesia – sampling and DNA analysis

- **The set of reference varieties may be considered as a representative sample of the genetic variation in the Freesia germplasm**

- **Sampling for DNA analysis**
  - Leaf material of 5 individual plants pooled.
  - All material delivered under code
- **DNA analysis**
  - Genotyping with AFLP using eight primer combinations resulted in 116 markers (presence/absence)

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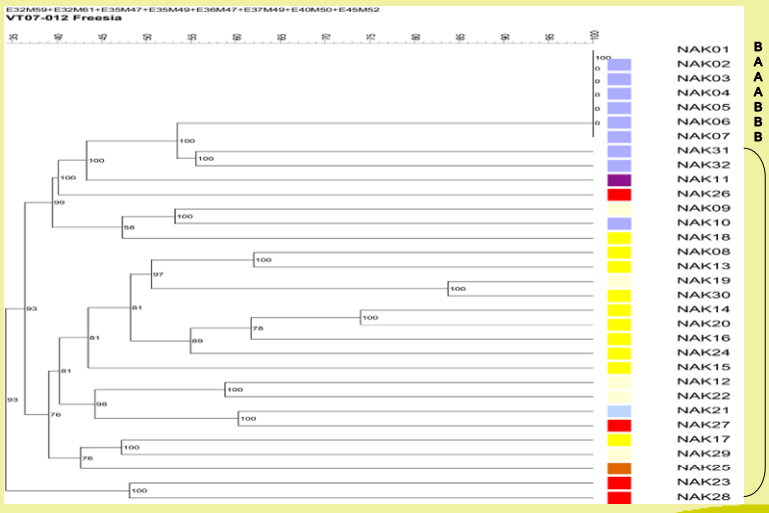
Freesia – Clustering analysis – Jaccard



Freesia - conclusions

- Genetic variation in total dataset is high
- The DNA profiles of all samples of variety A and variety B were identical (100%) under the conditions used
- The
- se results can be explained by either:
  - Variety A and B are identical, or
  - Variety B is essentially derived from Variety A

Freesia

- Study is used as evidence in court case
- Many arguments were considered
- The molecular results from this study contributed to the legal judgement that essentially derivation was proven

Final conclusions

- Morphological assessment and genetic diversity analysis act synergisticly
- DNA fingerprinting is very valuable in infringement and EDV matters

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