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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

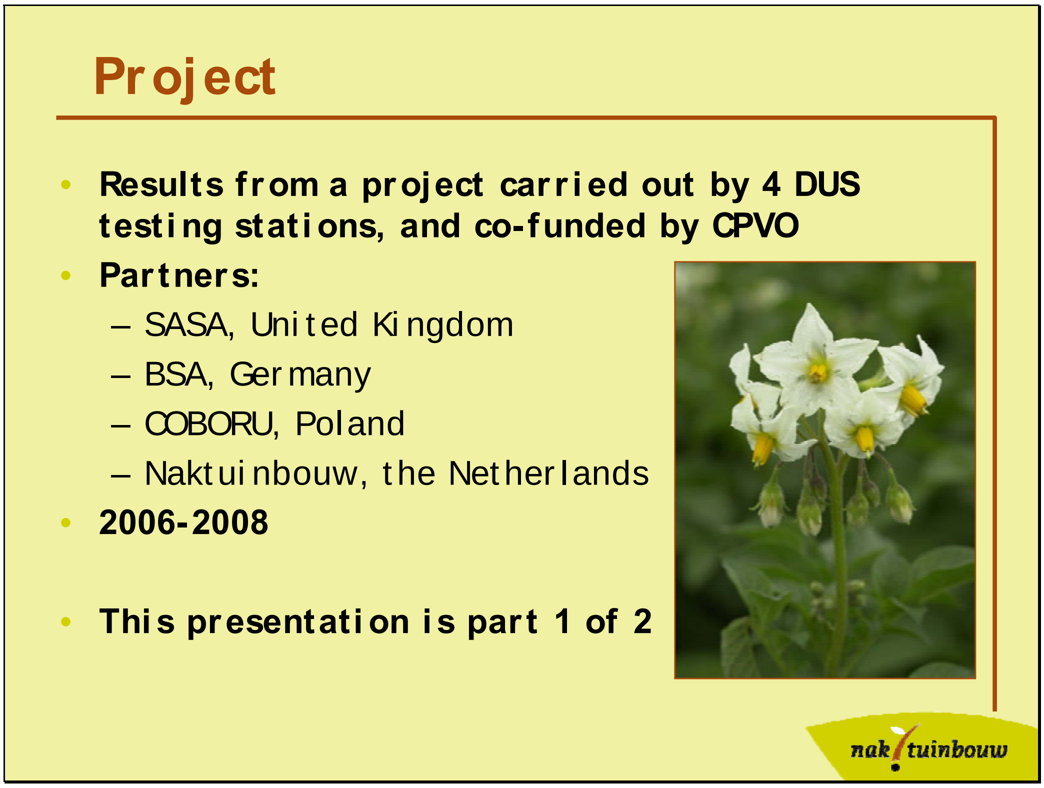
CONSTRUCTION OF AN INTEGRATED MICROSATELLITE AND KEY MORPHOLOGICAL CHARACTERISTIC DATABASE OF POTATO VARIETIES ON THE EU COMMON CATALOGUE
PART 1: DISCUSSION OF MORPHOLOGICAL AND MOLECULAR DATA

Document prepared by experts from the Netherlands

Construction of an integrated microsatellite and key morphological characteristic database of potato varieties on the EU common catalogue

Part 1: Discussion of morphological and molecular data

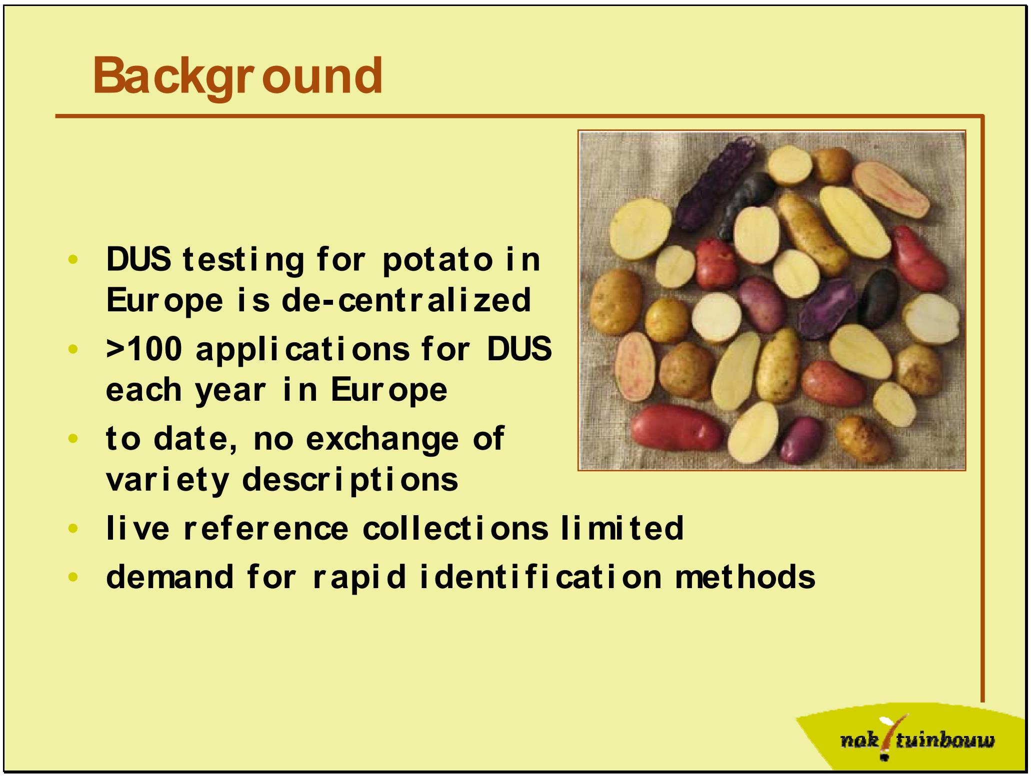
Lysbeth Hof



Project

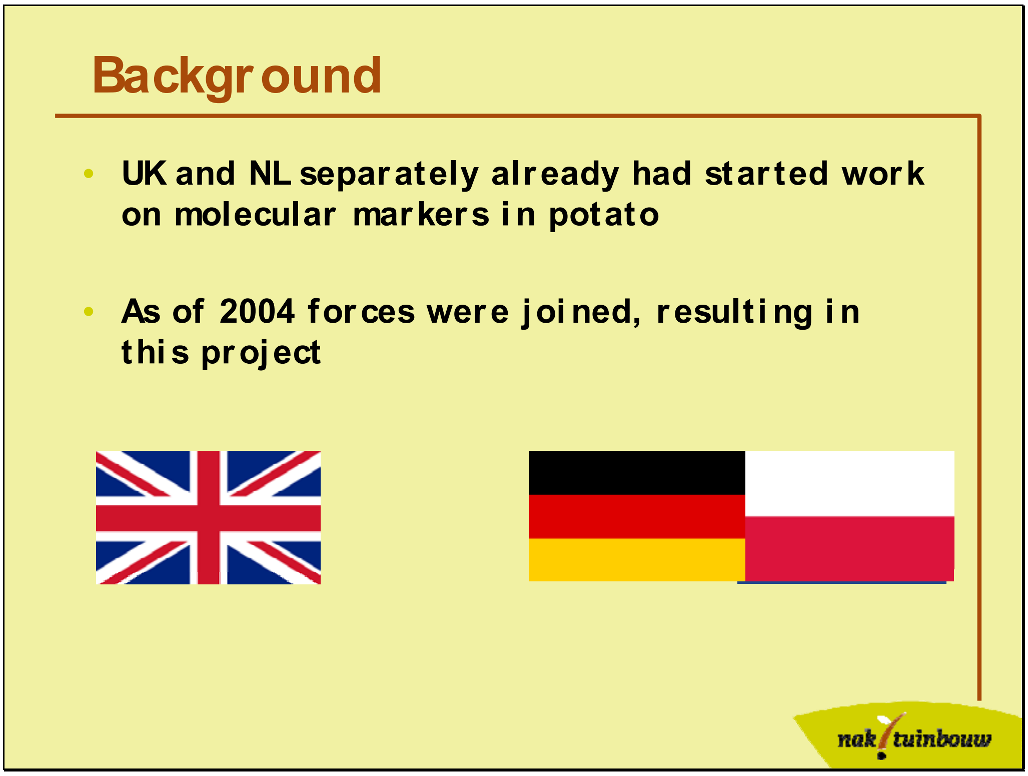
- Results from a project carried out by 4 DUS testing stations, and co-funded by CPVO
- Partners:
  - SASA, United Kingdom
  - BSA, Germany
  - COBORU, Poland
  - Naktuinbouw, the Netherlands
- 2006-2008

- This presentation is part 1 of 2



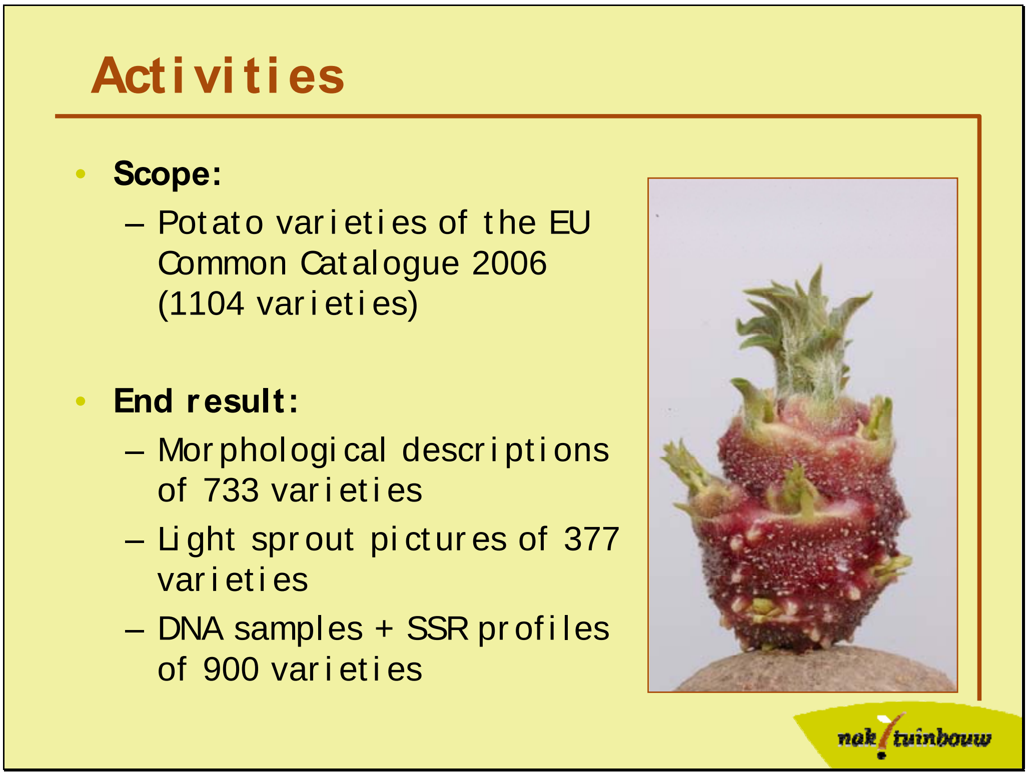
Background

- DUS testing for potato in Europe is de-centralized
- >100 applications for DUS each year in Europe
- to date, no exchange of variety descriptions
- live reference collections limited
- demand for rapid identification methods



Background

- UK and NL separately already had started work on molecular markers in potato
- As of 2004 forces were joined, resulting in this project



Activities

- **Scope:**
  - Potato varieties of the EU Common Catalogue 2006 (1104 varieties)
- **End result:**
  - Morphological descriptions of 733 varieties
  - Light sprout pictures of 377 varieties
  - DNA samples + SSR profiles of 900 varieties

Morphological descriptions

- **In total 856 descriptions of 733 varieties:**
  - – 622 from a single country
  - – 99 from 2 countries
  - – 11 from 3 countries
  - – 1 from all 4 countries

- **Characteristics:**
  - – light sprout char.
  - – TQ char. where available

Morphological descriptions

- **Guideline: TG/23/6, except UK: TG/23/5**
- **Both guidelines are different for some characteristics: for these the UK data were not used in comparisons**
- **Comparisons based on 12 varieties with descriptions of ≥ 3 countries**

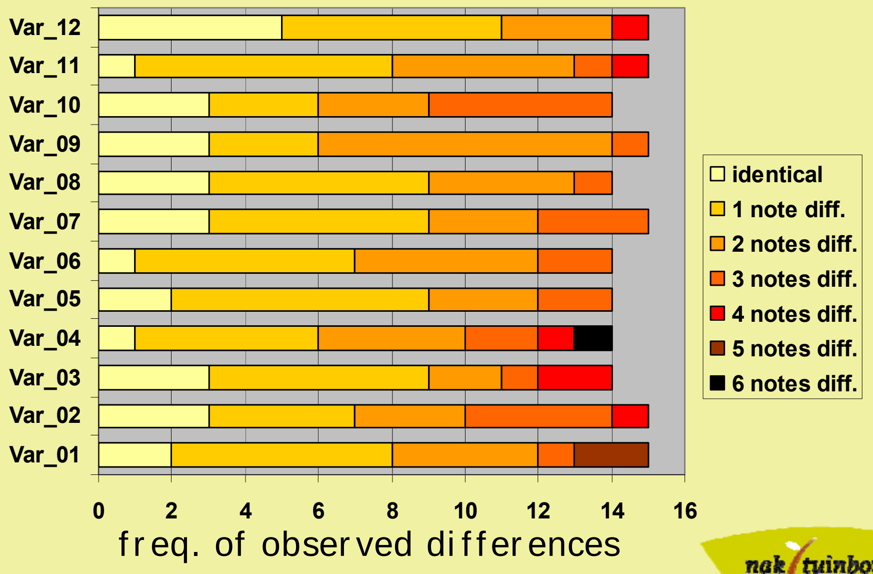
Morphological descriptions

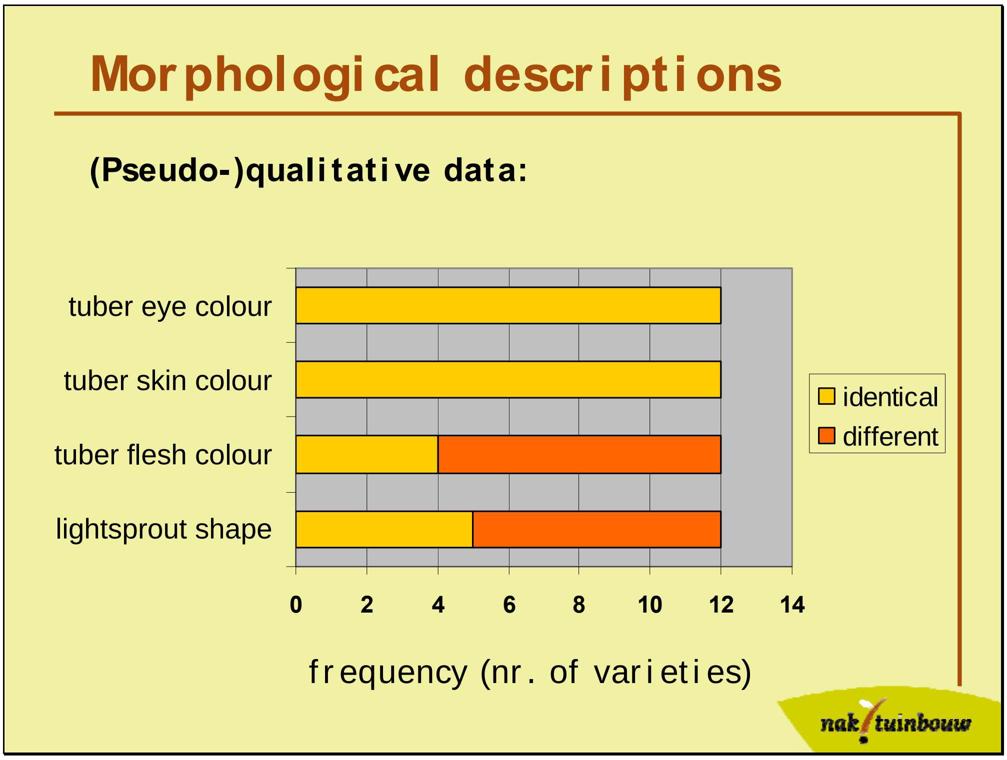
Quantitative data:

Var_xx	characteristic								
	1	3	4	5	6	7	8	9	10 …
Country A	3	7	3	2	3	8	9	2	5
Country B	3	6	2	5	3	8	8	5	6
Country C	4	5	-	4	3	-	8	3	4
difference	1	2	1	3	0	0	1	3	2

Morphological descriptions

Quantitative data (15 char.):





Morphological descriptions

(Pseudo-)qualitative data:

Morphological descriptions

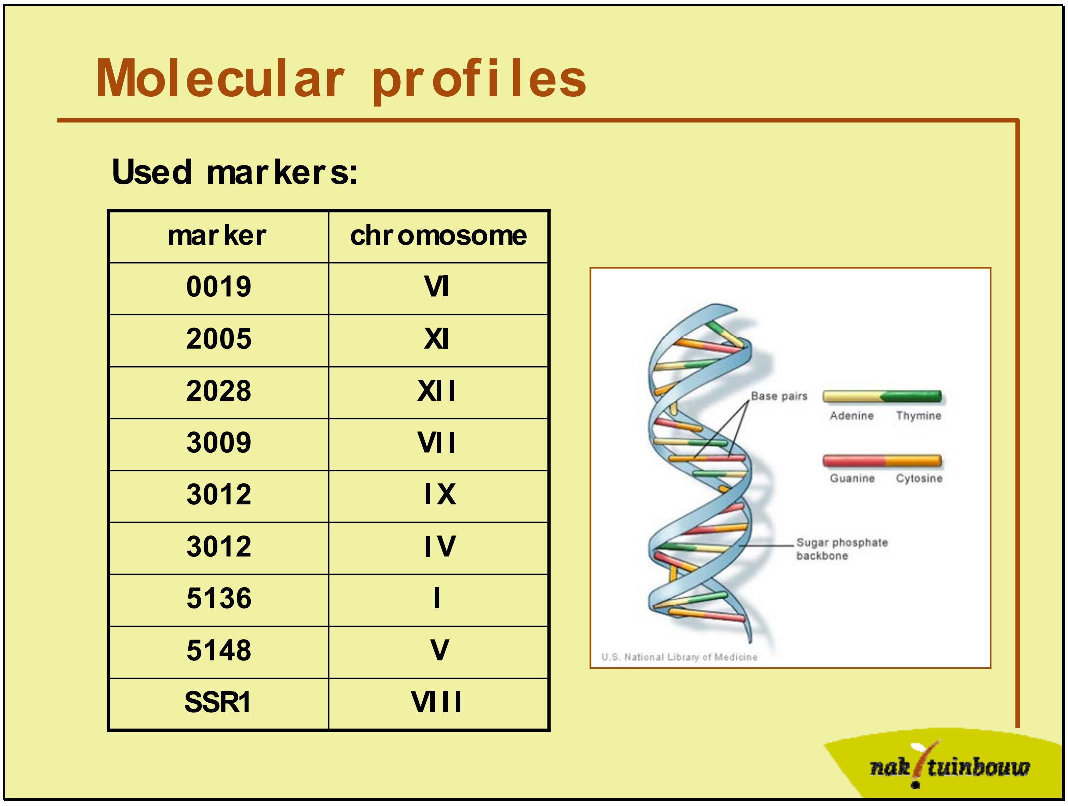
- **Conclusions:**
  - Descriptions vary between different sources (countries)
  - Descriptions of the same variety from different sources would have been declared distinct in almost all cases
  - Exchange of descriptions between offices is not useful for reference collections
  - Comparisons should be carried out side-by-side

nak tuinbouw

Molecular profiles

- **In total 900 varieties**
  - samples collected from own reference collections
  - samples obtained from breeders/maintainers

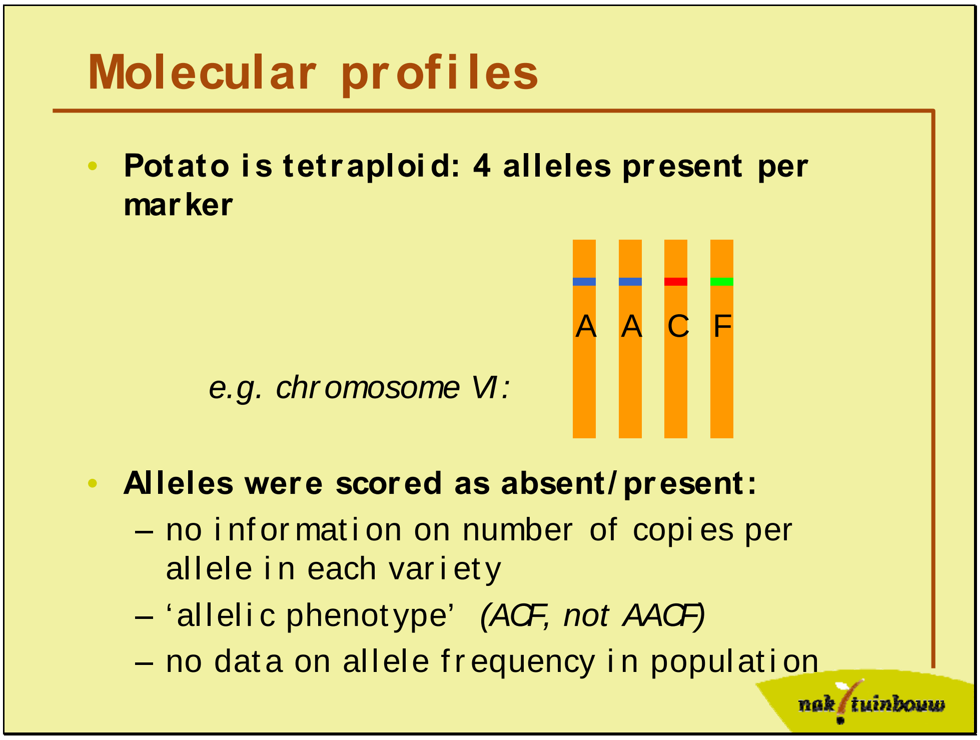
- **More than 200 varieties with samples from more than 1 source**
- **Most varieties analysed in 2 labs (NL and UK)**
- **Many varieties 2 profiles per lab (UK)**
- **In case of anomalies/doubts: variety re-sampled**
- **=> in total almost 3000 profiles scored**



Molecular profiles

Used markers:

marker	chromosome
0019	VI
2005	XI
2028	XII
3009	VII
3012	IX
3012	IV
5136	I
5148	V
SSR1	VIII

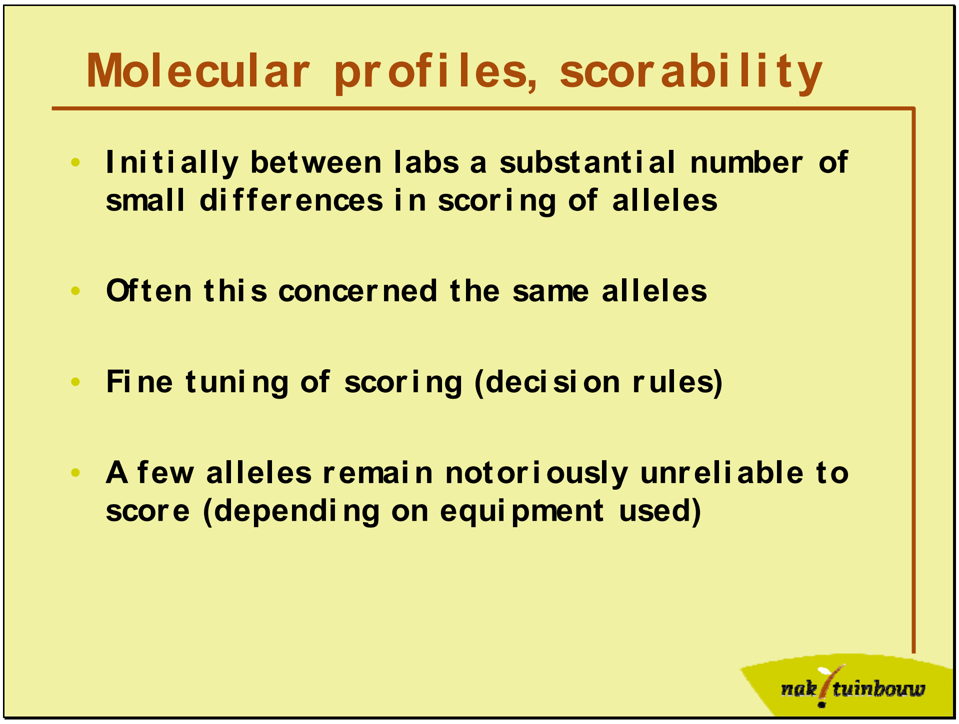


Molecular profiles

- **Potato is tetraploid: 4 alleles present per marker**

e.g. chromosome VI:

- **Alleles were scored as absent/present:**
  - – no information on number of copies per allele in each variety
  - – ‘allelic phenotype’ *(ACF, not AACF)*
  - – no data on allele frequency in population



Molecular profiles, scorability

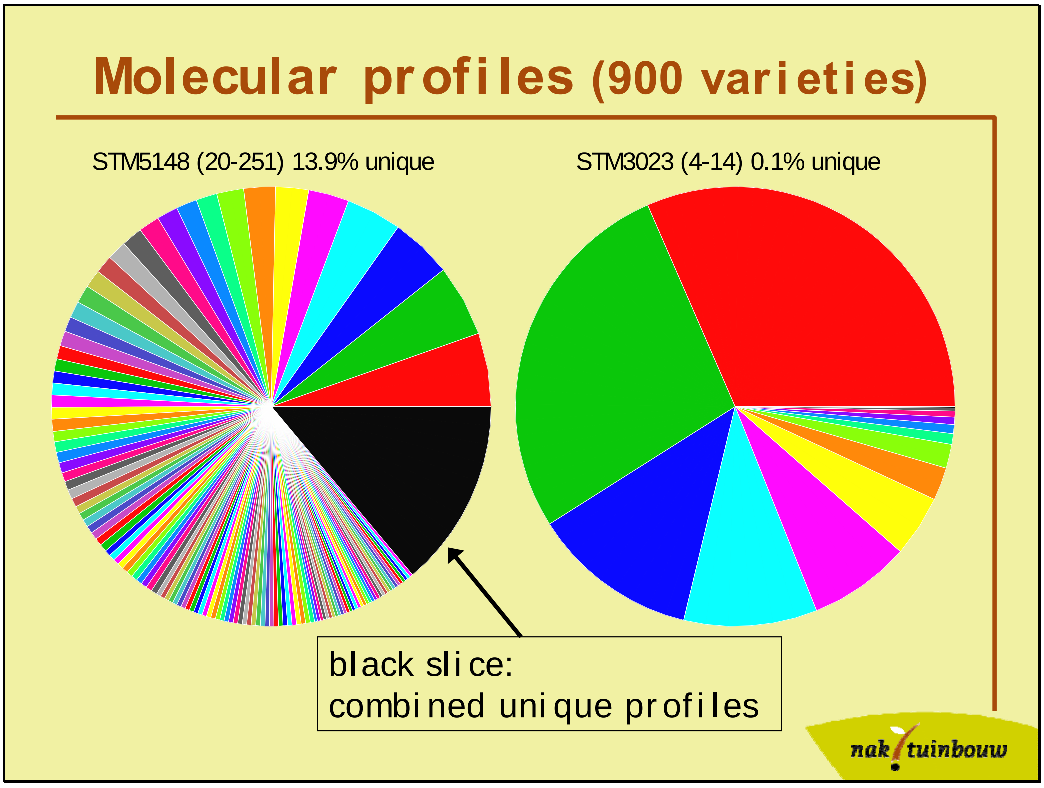
- **Initially between labs a substantial number of small differences in scoring of alleles**
- **Often this concerned the same alleles**
- **Fine tuning of scoring (decision rules)**
- **A few alleles remain notoriously unreliable to score (depending on equipment used)**

Molecular profiles, scorability

- With multiple samples/analyses consensus on correct profile can be reached
- Discarding these alleles in analyses has no effect on effectiveness of database
- When constructing a database: analyses at 2 labs enhances robustness

Molecular profiles (900 varieties)

marker	# alleles	# different profiles	% unique profiles	% most common profile	PIC value
0019	10	61	1.8	17	0.92
2005	6	21	0.4	37	0.80
2028	9	62	2.2	23	0.90
3009	14	48	2.1	34	0.81
3012	7	27	0.2	19	0.87
3023	4	14	0.1	32	0.79
5136	11	54	2.8	14	0.92
5148	20	251	13.9	5	0.98
SSR1	14	119	5.5	17	0.93



Molecular profiles (900 varieties)

STM5148 (20-251) 13.9% unique

STM3023 (4-14) 0.1% unique

black slice:
combined unique profiles

Molecular profiles (900 varieties)

- **Apart from known mutants, 8 pairs with identical profile were found:**
  - 1 already suspected being the same
  - 1 already suspected being mislabeled
  - 1 already suspected being mutant, not offspring as was claimed
  - 5 unexplained
    - all pairs have very similar morphological descriptions
    - material re-sample, identical results
    - another 31 markers used, identical results

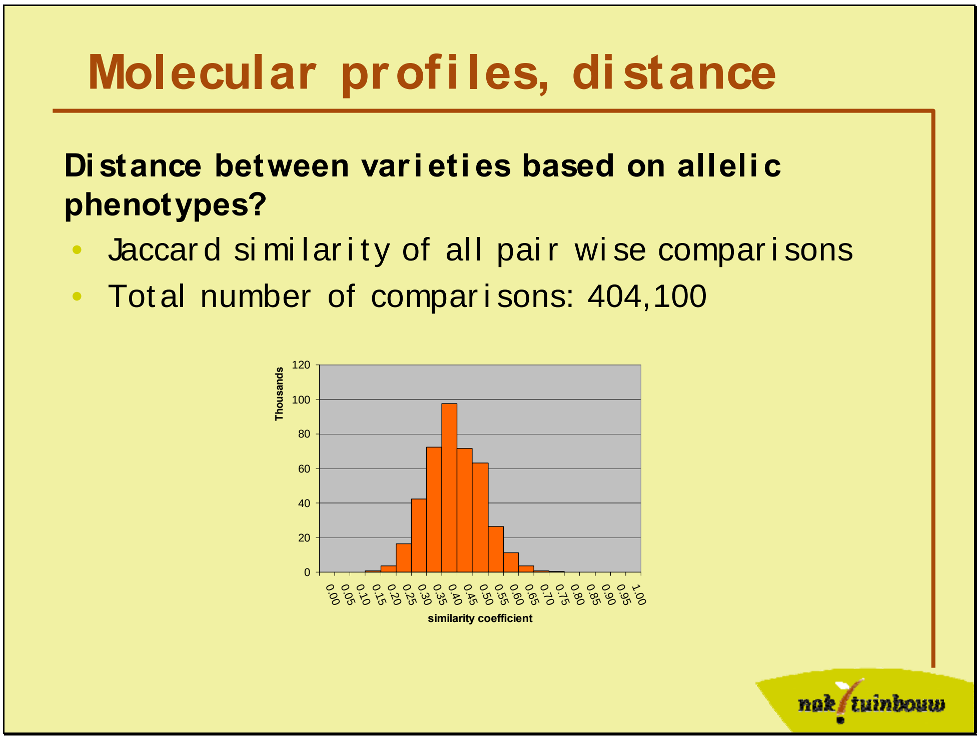
Molecular profiles, probability

Probability of 2 unrelated varieties showing identical profile by chance?

- No allele frequencies available
- Based on most common allelic phenotype per marker:

1 in 2.8 million

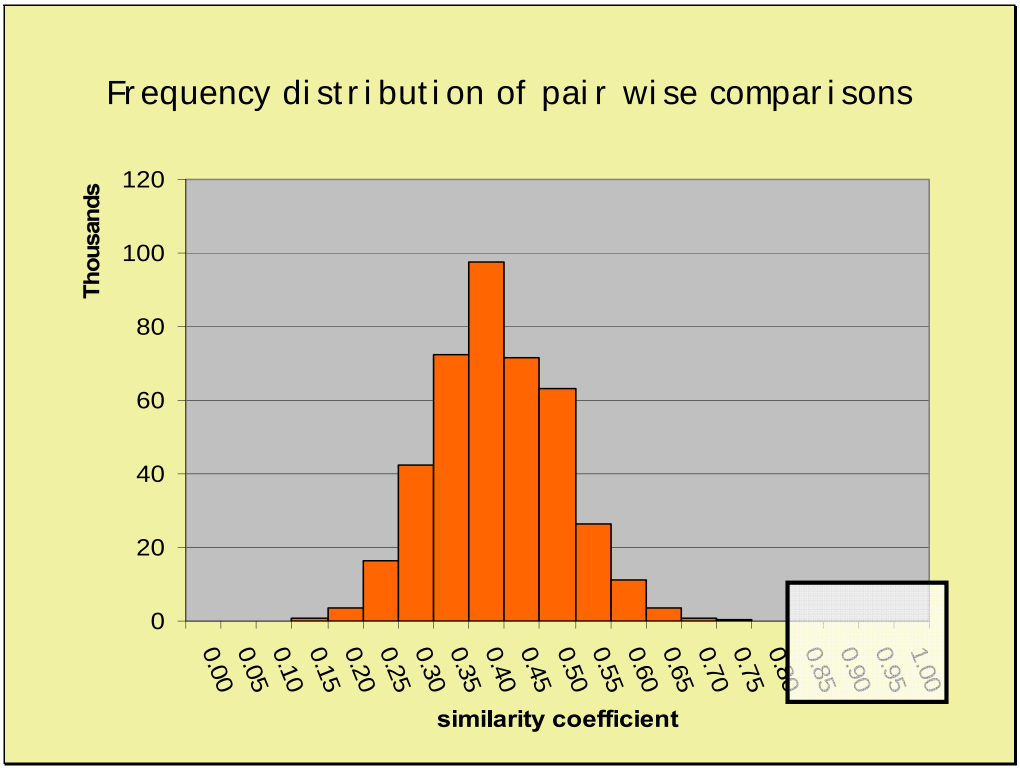
- (Common ancestry not taken into account)

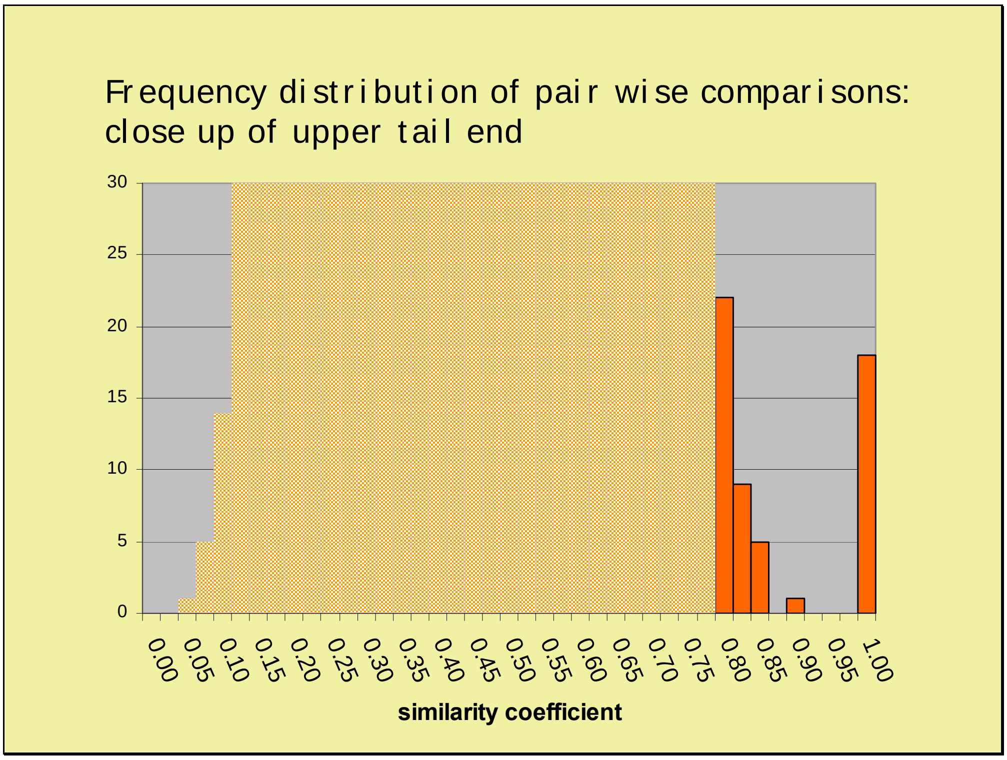


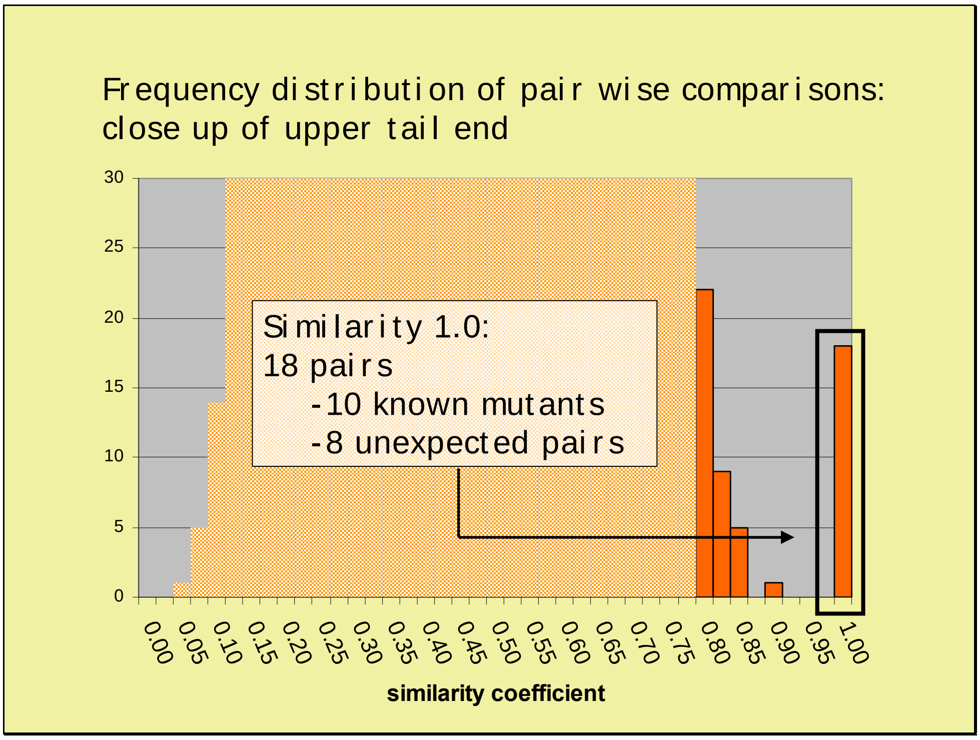
Molecular profiles, distance

Distance between varieties based on allelic phenotypes?

- Jaccard similarity of all pair wise comparisons
- Total number of comparisons: 404,100

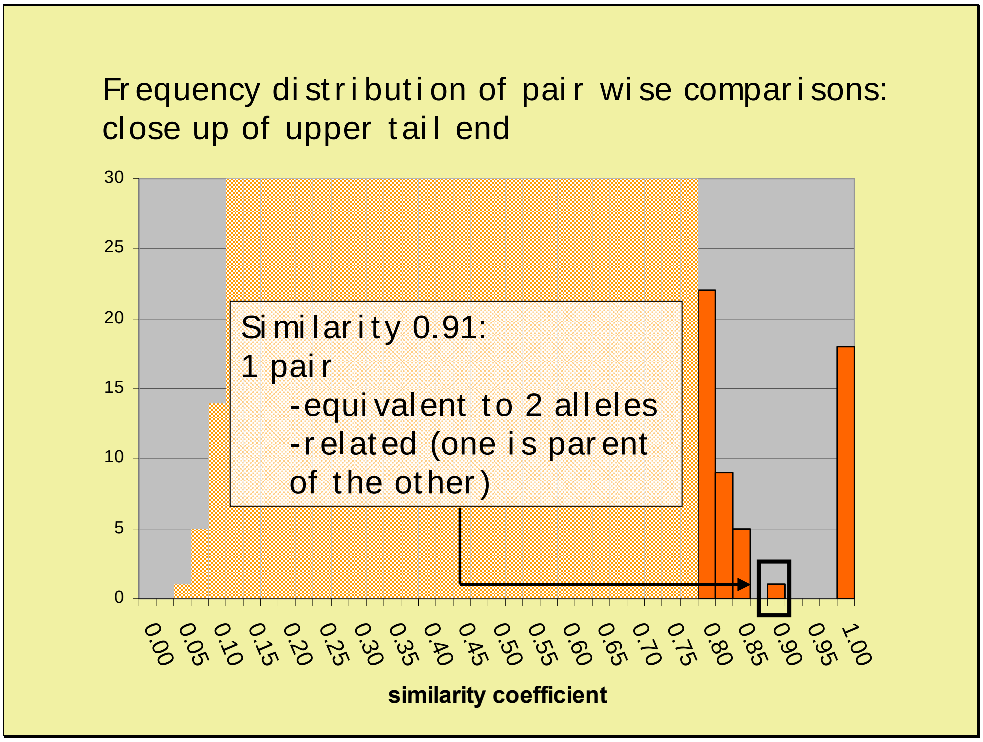

Frequency distribution of pair wise comparisons
Thousands
120
100
80
60
40
20
0
0.00
0.05
0.10
0.15
0.20
0.25
0.30
0.35
0.40
0.45
0.50
0.55
0.60
0.65
0.70
0.75
0.80
0.85
0.90
0.95
1.00
similarity coefficient


Frequency distribution of pair wise comparisons: close up of upper tail end
30
25
20
15
10
5
0
0.00
0.05
0.10
0.15
0.20
0.25
0.30
0.35
0.40
0.45
0.50
0.55
0.60
0.65
0.70
0.75
0.80
0.85
0.90
0.95
1.00
similarity coefficient



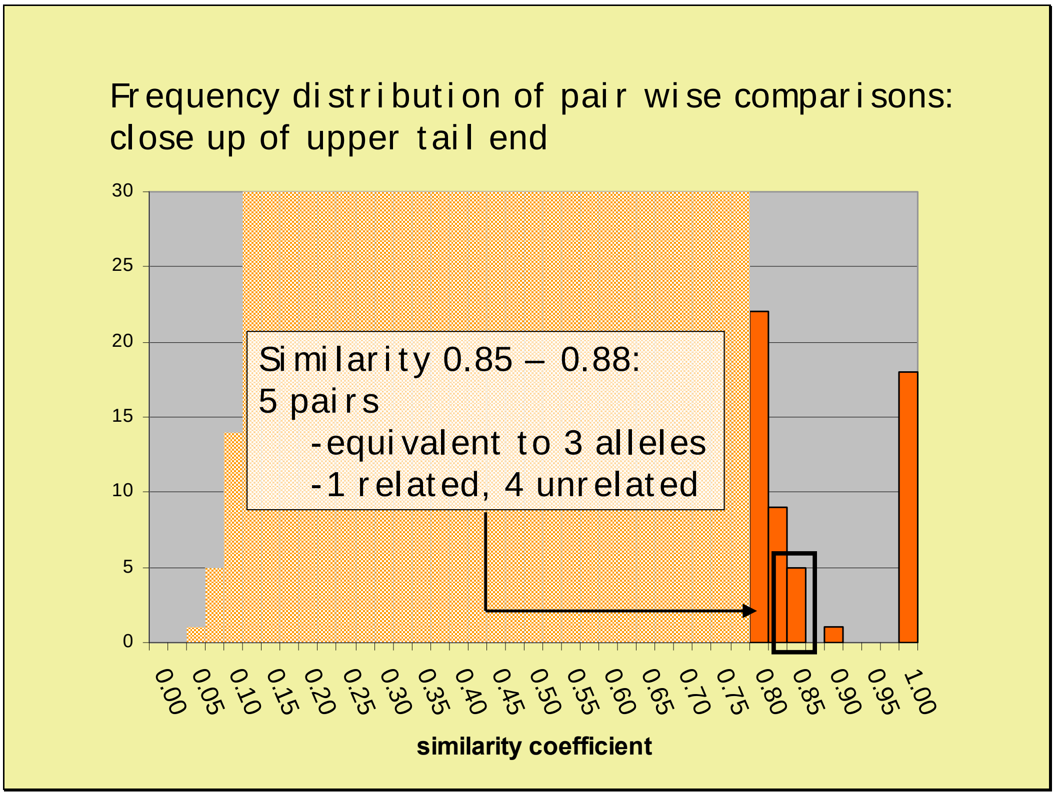
Frequency distribution of pair wise comparisons: close up of upper tail end

Similarity 1.0:
18 pairs
- 10 known mutants
- 8 unexpected pairs



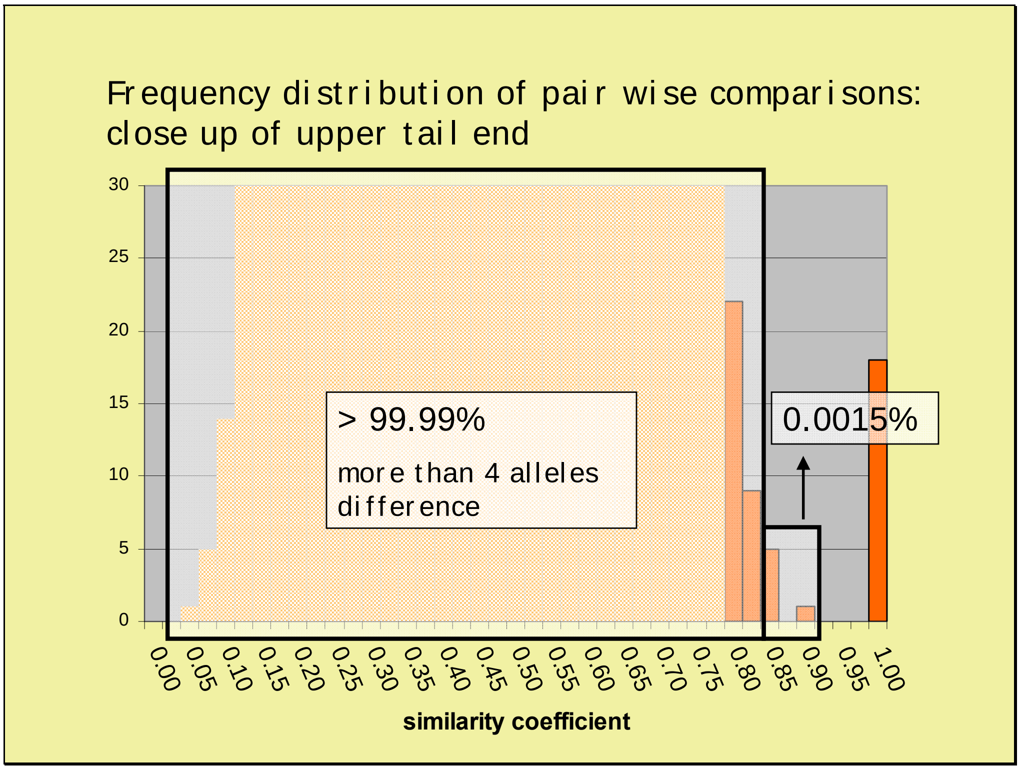
Frequency distribution of pair wise comparisons: close up of upper tail end

Similarity 0.91:
1 pair
- equivalent to 2 alleles
- related (one is parent of the other)



Frequency distribution of pair wise comparisons: close up of upper tail end

Similarity 0.85 – 0.88:
5 pairs
-equivalent to 3 alleles
-1 related, 4 unrelated

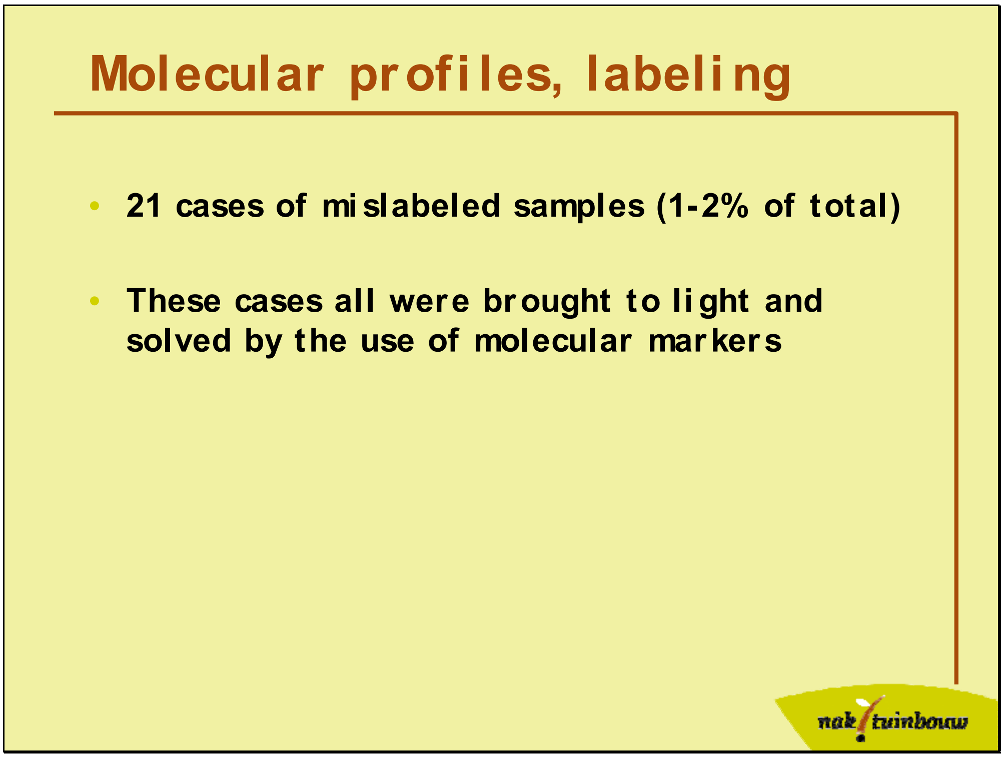


Frequency distribution of pair wise comparisons: close up of upper tail end

> 99.99%

more than 4 alleles difference

0.0015%

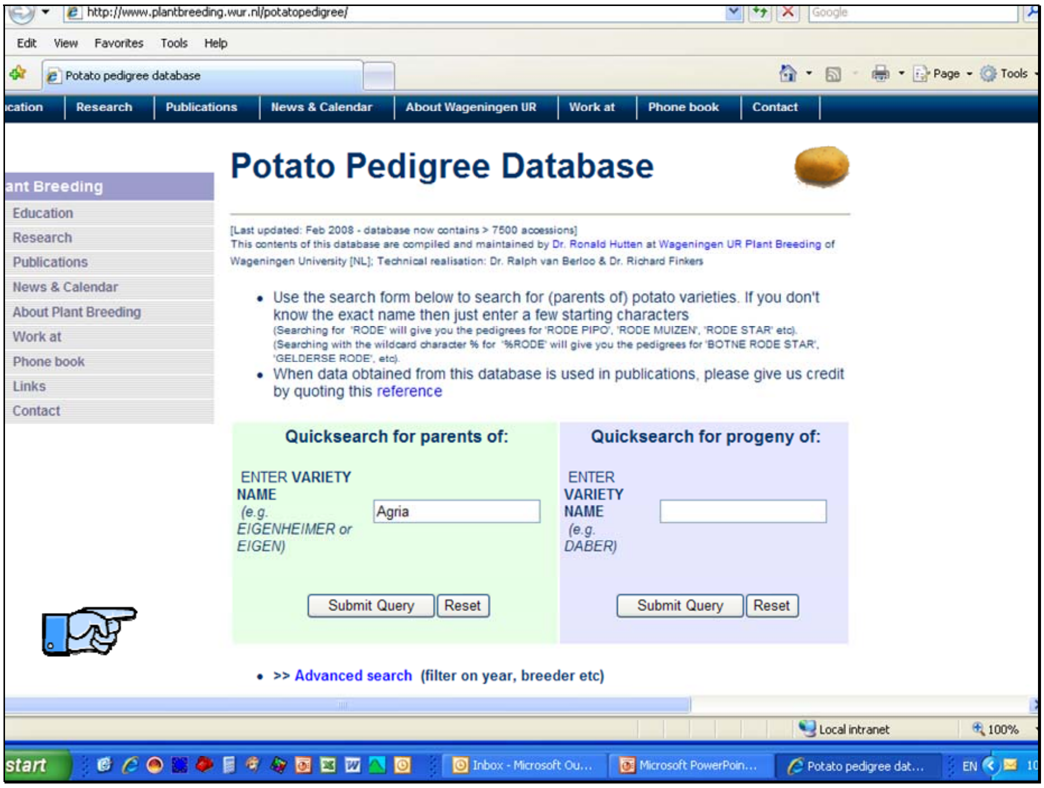


Molecular profiles, labeling

- **21 cases of mislabeled samples (1-2% of total)**
- **These cases all were brought to light and solved by the use of molecular markers**

Molecular profiles, denomination

- **Same variety name - different profiles?**
  - variety names are re-used
  - old varieties from before regulations on denomination
  - mislabeling
  - help from pedigree-information
    - *www.plantbreeding.wur.nl/potatopedigree*
    - *www.europotato.org*



Potato Pedigree Database

[Last updated: Feb 2008 - database now contains > 7500 accessions]
This contents of this database are compiled and maintained by Dr. Ronald Hutten at Wageningen UR Plant Breeding of Wageningen University [NL]; Technical realisation: Dr. Ralph van Berloo & Dr. Richard Finkers

- Use the search form below to search for (parents of) potato varieties. If you don't know the exact name then just enter a few starting characters
  (Searching for 'RODE' will give you the pedigrees for 'RODE PIPO', 'RODE MUIZEN', 'RODE STAR' etc).
  (Searching with the wildcard character % for '%RODE' will give you the pedigrees for 'BOTNE RODE STAR', 'GELDERSE RODE', etc).
- When data obtained from this database is used in publications, please give us credit by quoting this reference

Quicksearch for parents of:

ENTER **VARIETY NAME** *(e.g. EIGENHEIMER or EIGEN)* Agria

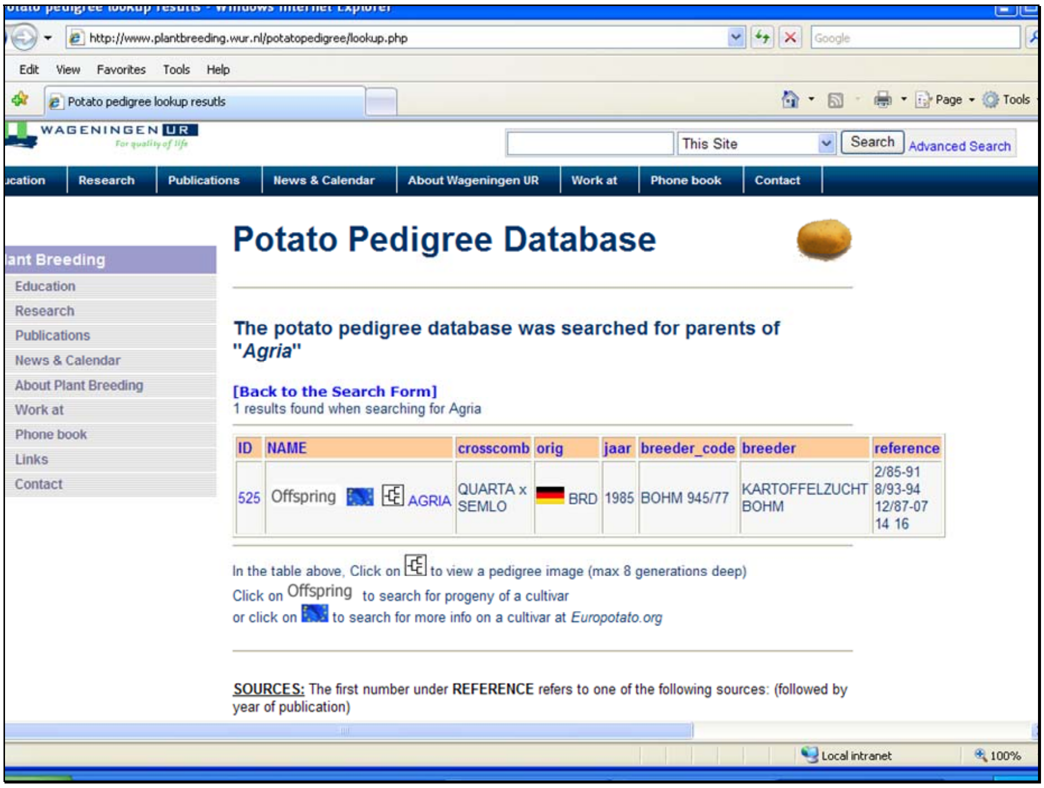
Submit Query | Reset

Quicksearch for progeny of:

ENTER **VARIETY NAME** *(e.g. DABER)*

Submit Query | Reset

- **>> Advanced search (filter on year, breeder etc)**



Potato Pedigree Database

The potato pedigree database was searched for parents of "*Agria*"

[Back to the Search Form]
1 results found when searching for Agria

ID	NAME	crosscomb	orig	jaar	breeder_code	breeder	reference
525	Offspring AGRIA	QUARTA x SEMLO	BRD	1985	BOHM 945/77	KARTOFFELZUCHT BOHM	2/85-91 8/93-94 12/87-07 14 16

In the table above, Click on to view a pedigree image (max 8 generations deep)
Click on Offspring to search for progeny of a cultivar
or click on to search for more info on a cultivar at *Europotato.org*

<u>**SOURCES:**</u> The first number under **REFERENCE** refers to one of the following sources: (followed by year of publication)

The European Cultivated Potato Database

Quick search ...

Enter the first four letters of a variety name . . .

AGRI

. . . then press Submit

Submit

[New search]

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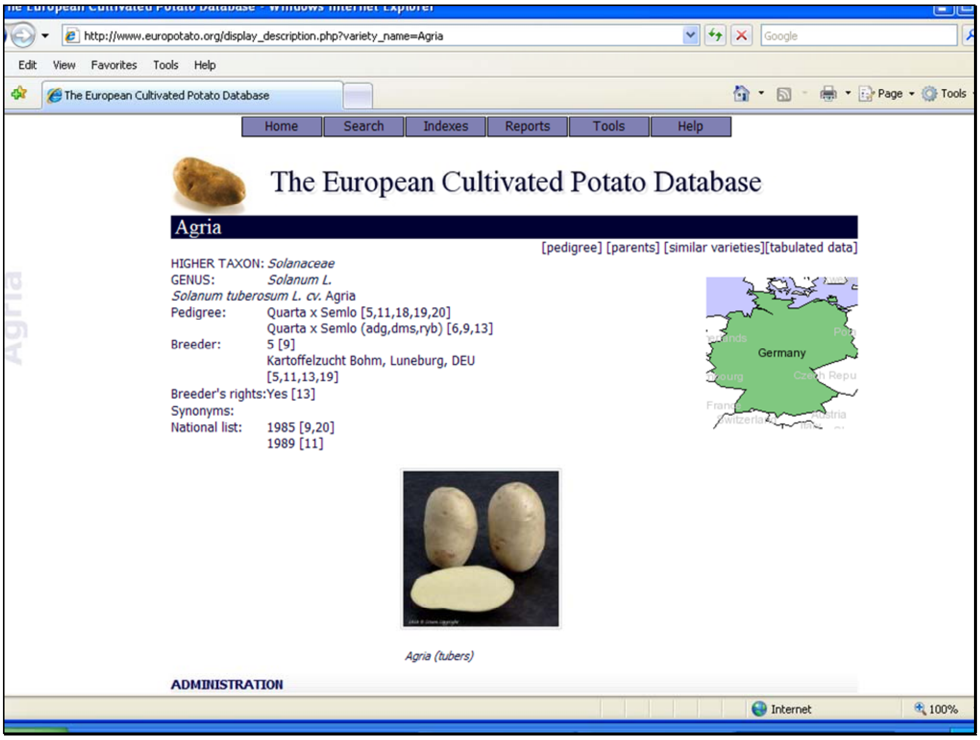


The European Cultivated Potato Database

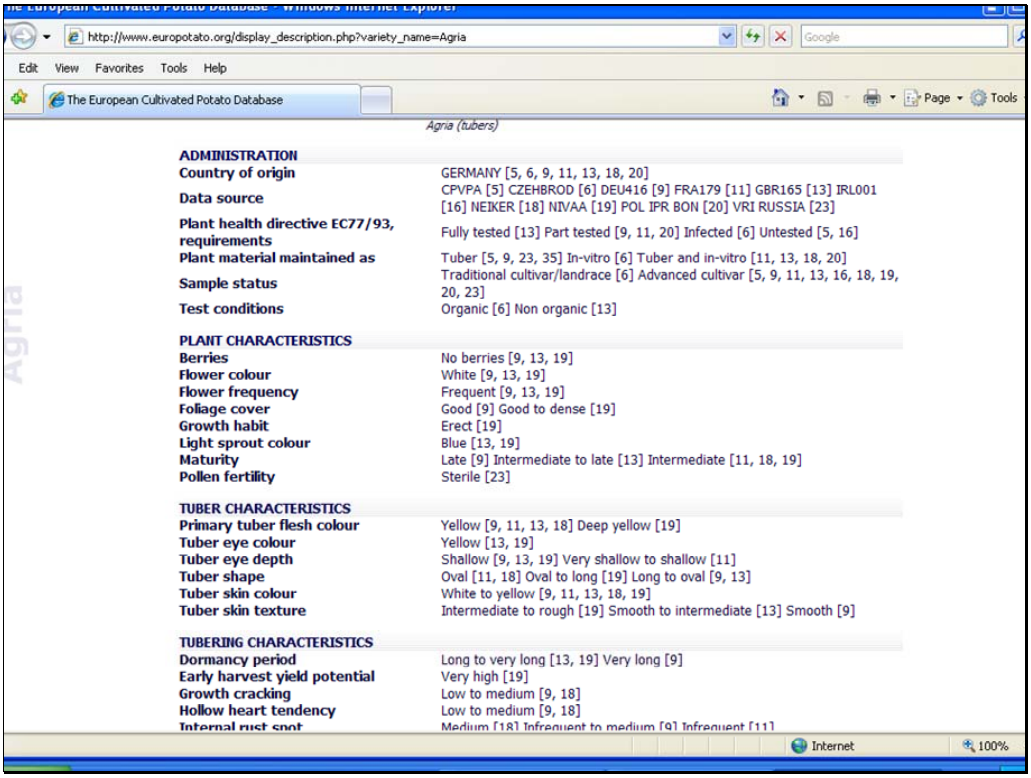
Agria

[pedigree] [parents] [similar varieties][tabulated data]

HIGHER TAXON: *Solanaceae*
GENUS: *Solanum L.*
Solanum tuberosum L. cv. Agria
Pedigree: Quarta x Semlo [5,11,18,19,20]
Quarta x Semlo (adg,dms,ryb) [6,9,13]
Breeder: 5 [9]
Kartoffelzucht Bohm, Luneburg, DEU
[5,11,13,19]
Breeder's rights: Yes [13]
Synonyms:
National list: 1985 [9,20]
1989 [11]

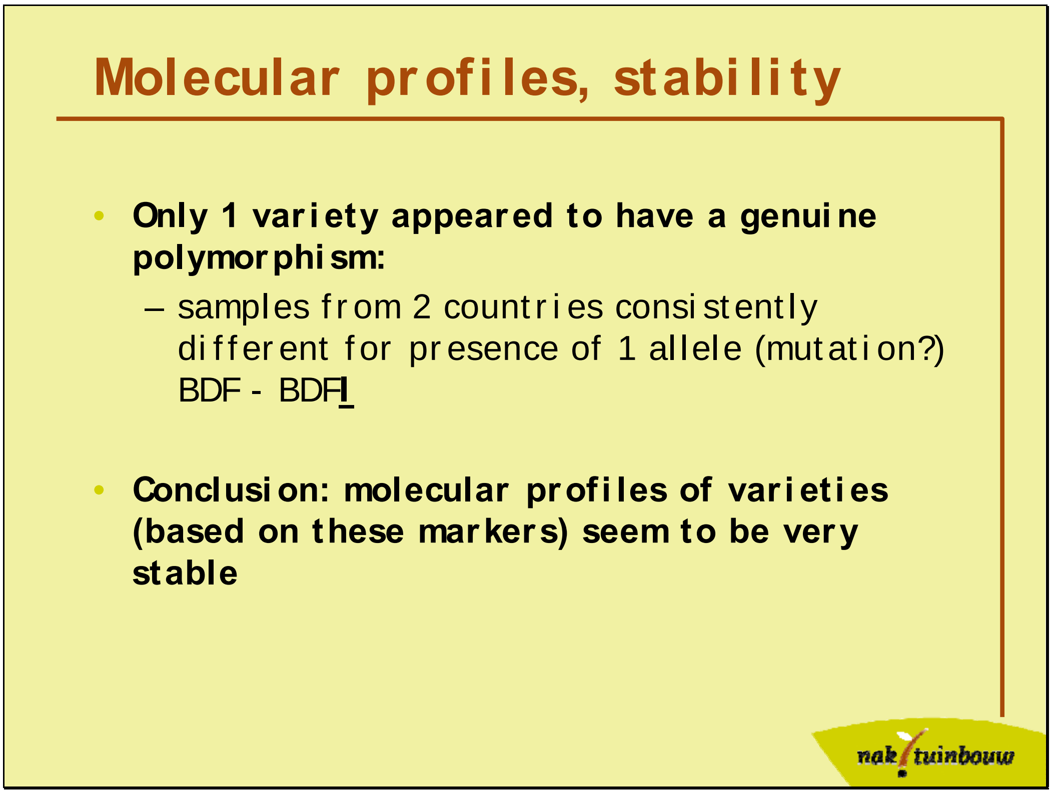
Agria (tubers)

ADMINISTRATION



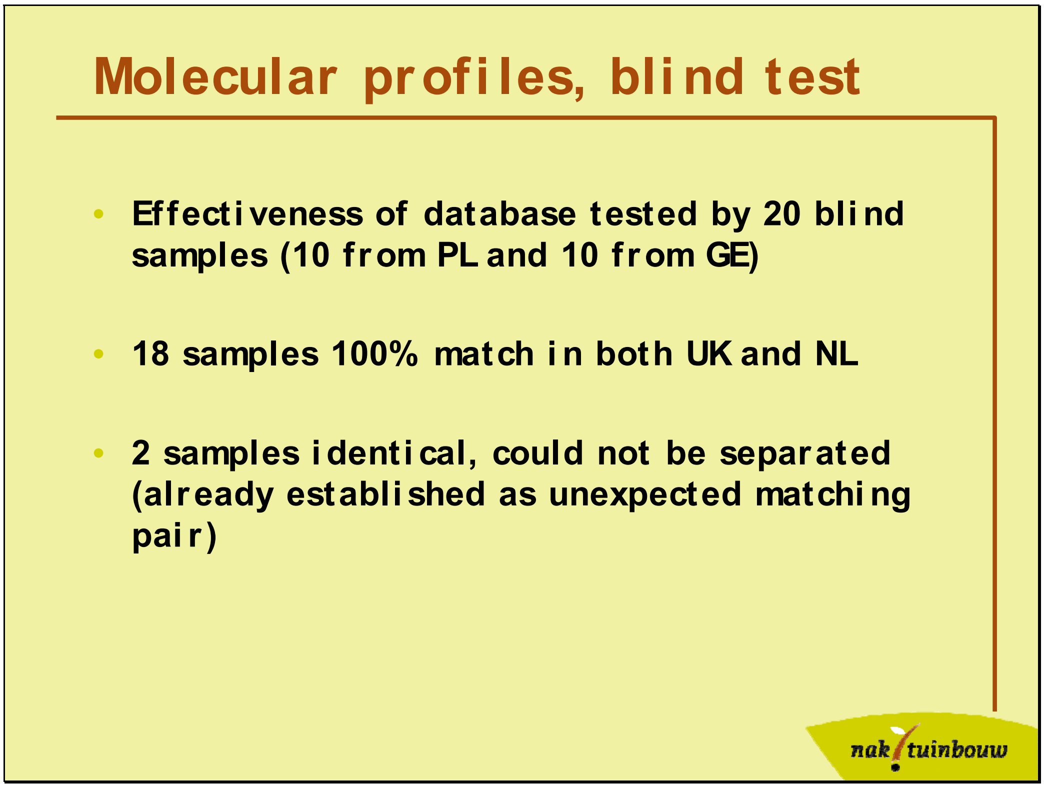
Agria (tubers)

ADMINISTRATION	
Country of origin	GERMANY [5, 6, 9, 11, 13, 18, 20]
Data source	CPVPA [5] CZEHBROD [6] DEU416 [9] FRA179 [11] GBR165 [13] IRL001 [16] NEIKER [18] NIVAA [19] POL IPR BON [20] VRI RUSSIA [23]
Plant health directive EC77/93, requirements	Fully tested [13] Part tested [9, 11, 20] Infected [6] Untested [5, 16]
Plant material maintained as	Tuber [5, 9, 23, 35] In-vitro [6] Tuber and in-vitro [11, 13, 18, 20]
Sample status	Traditional cultivar/landrace [6] Advanced cultivar [5, 9, 11, 13, 16, 18, 19, 20, 23]
Test conditions	Organic [6] Non organic [13]
PLANT CHARACTERISTICS	
Berries	No berries [9, 13, 19]
Flower colour	White [9, 13, 19]
Flower frequency	Frequent [9, 13, 19]
Foliage cover	Good [9] Good to dense [19]
Growth habit	Erect [19]
Light sprout colour	Blue [13, 19]
Maturity	Late [9] Intermediate to late [13] Intermediate [11, 18, 19]
Pollen fertility	Sterile [23]
TUBER CHARACTERISTICS	
Primary tuber flesh colour	Yellow [9, 11, 13, 18] Deep yellow [19]
Tuber eye colour	Yellow [13, 19]
Tuber eye depth	Shallow [9, 13, 19] Very shallow to shallow [11]
Tuber shape	Oval [11, 18] Oval to long [19] Long to oval [9, 13]
Tuber skin colour	White to yellow [9, 11, 13, 18, 19]
Tuber skin texture	Intermediate to rough [19] Smooth to intermediate [13] Smooth [9]
TUBERING CHARACTERISTICS	
Dormancy period	Long to very long [13, 19] Very long [9]
Early harvest yield potential	Very high [19]
Growth cracking	Low to medium [9, 18]
Hollow heart tendency	Low to medium [9, 18]
Internal rust spot	Medium [18] Infrequent to medium [9] Infrequent [11]



Molecular profiles, stability

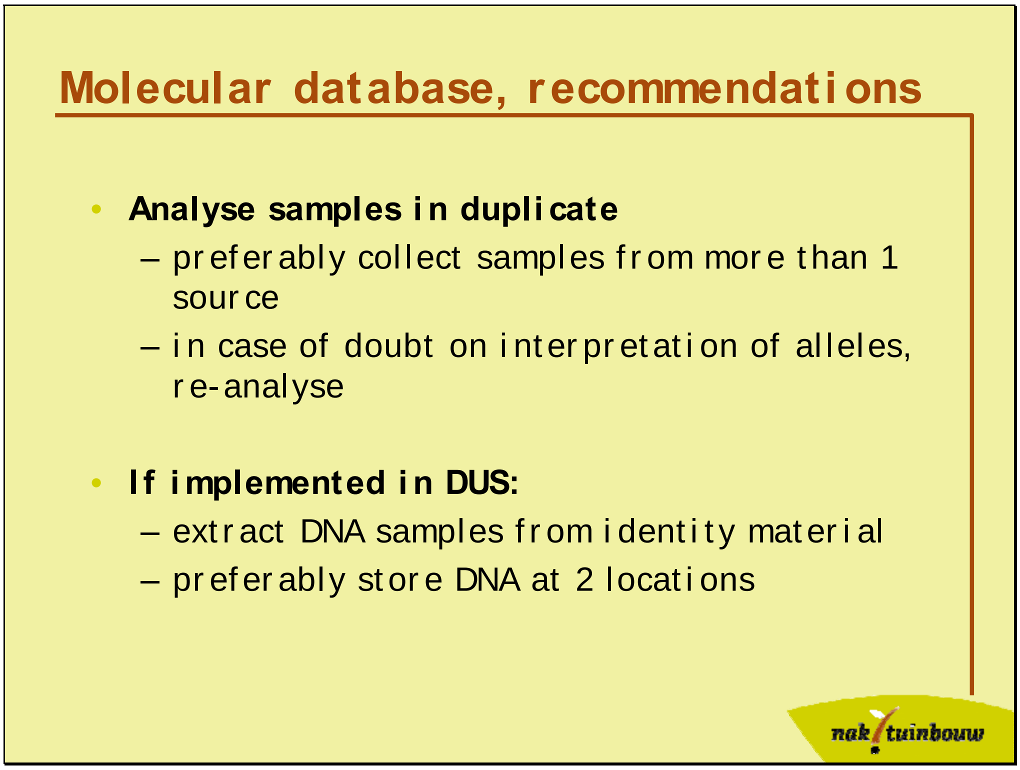
- **Only 1 variety appeared to have a genuine polymorphism:**
  - samples from 2 countries consistently different for presence of 1 allele (mutation?) BDF - BDFI
- **Conclusion: molecular profiles of varieties (based on these markers) seem to be very stable**



Molecular profiles, blind test

- Effectiveness of database tested by 20 blind samples (10 from PL and 10 from GE)
- 18 samples 100% match in both UK and NL
- 2 samples identical, could not be separated (already established as unexpected matching pair)

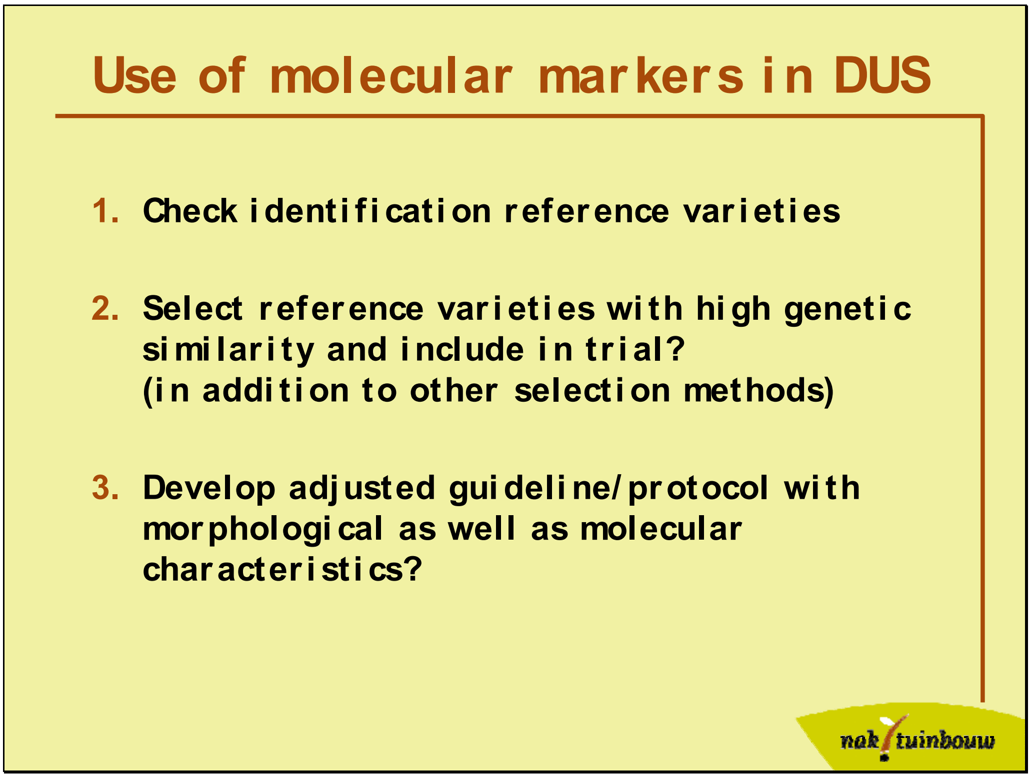
nak tuinbouw



Molecular database, recommendations

- **Analyse samples in duplicate**
  - preferably collect samples from more than 1 source
  - in case of doubt on interpretation of alleles, re-analyse
- **If implemented in DUS:**
  - extract DNA samples from identity material
  - preferably store DNA at 2 locations

nak tuinbouw



Use of molecular markers in DUS

1. Check identification reference varieties
2. Select reference varieties with high genetic similarity and include in trial?
   (in addition to other selection methods)
3. Develop adjusted guideline/protocol with morphological as well as molecular characteristics?

Co-workers

- Alex Reid
- Beate Rücker
- Swenja Tamms
- Ewa Milczynska
- Bogna Kowalczyk
- staff of PRI (NL)

THANK YOU FOR YOUR ATTENTION!

Quality in Horticulture

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