

## Content



- DNA in Variety Protection:  
PVP/PBR – DUS examination



- Genetic Analyses - Infringements of PVP  
(Variety Tracer)



# DNA in Plant Variety Protection DUS examination

## Molecular markers in DUS



### UPOV-BMT

- The Working Group on Biochemical and Molecular Techniques and DNA-profiling in particular (BMT)



Open group of DUS experts, biochemical and molecular specialists and plant breeders, that consider new techniques for application of molecular markers in DUS testing

## Molecular Markers in DUS



### • UPOV-BMT

*The Working Group on Biochemical and Molecular Techniques and DNA-profiling in particular (BMT)*



MODEL 1: Characteristic-specific molecular markers

MODEL 2: Combining phenotypic and molecular distances and in the management of variety collections



MODEL 3: Calibrated molecular distances in the management of variety collections

MODEL 4: Use of molecular marker characteristics

# BMT MODEL: 1



**Model 1:** Molecular Characteristics as Predictors of 'Traditional' Characteristics



a) Gene-specific markers for predicting individual phenotypic characteristics. (Reliable linkage between the marker and the expression of the characteristic required)  
- e.g. *disease resistance*



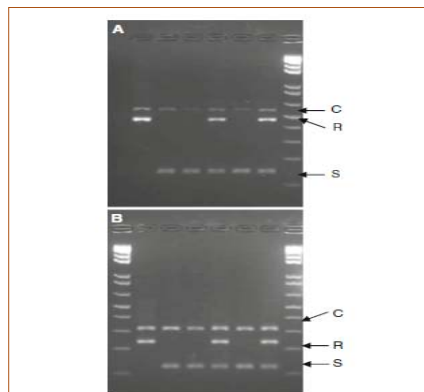
b) Use of a set of molecular characteristics which can be used to reliably estimate traditional characteristics; e.g. *quantitative trait loci (QTL)*

# MODEL 1: predictor for resistance



**Example**

*Verticillium* resistance in tomato



**Fig. 1** Example of molecular assay: results of *Verticillium* assays on test set for robustness assessment (2% agarose). Varieties are Campeon, Marmande, MoneyMaker, Persica, Kamonium and Chablis. **a** Ve1 assay: C: control band (580 bp), R: resistant allele (476 bp), S: susceptible allele (158 bp) **b** Yc2 assay: C: control band (321 bp), R: resistant allele (242 bp), S: susceptible allele (131 bp)

Third Appl. Genet.  
DOI 10.1007/s00122-009-1183-2

ORIGINAL PAPER

Development and evaluation of robust molecular markers linked to disease resistance in tomato for distinctness, uniformity and stability testing

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## BMT MODEL: 2



- **Model 2:** Combining phenotypic and molecular distances and in the management of variety collections
- Overall genetic assessment in relation to overall morphological assessment

## BMT MODEL: 2

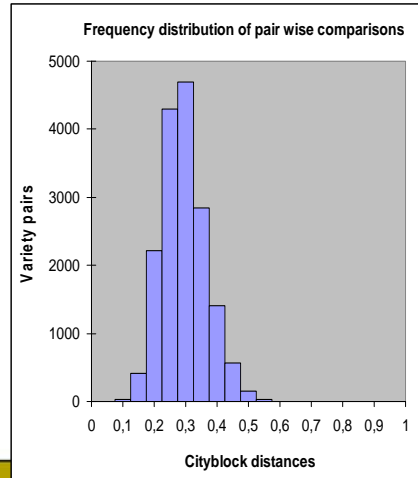


### Combining phenotypic and molecular distances management of variety collections model system POTATO

#### AIM:

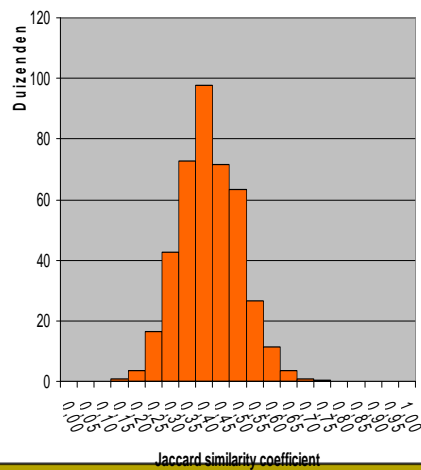
- Better selection of relevant references in field trial
- Exclude non-relevant reference varieties from field trial
- Reduce the number of reference varieties in the field trial
- Reduce the duration of the field trial
- Reduce costs

## MODEL 2: Phenotypic distance



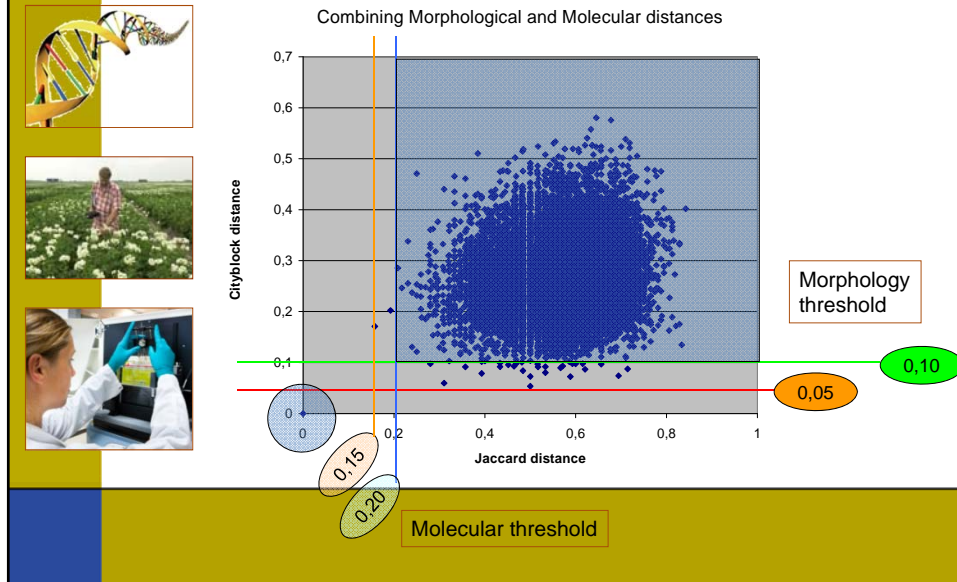
- 183 varieties
- 16.653 combinations
- Lightsprout and field characteristics
- Only quantitative characteristics
- Cityblock distance: sum of all differences

## MODEL 2: Genotypic distance

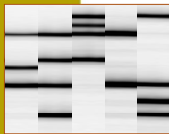


- 900 varieties under which the 183
- 404100 combinations
- Tubers from several origins investigated by two labs
- 9 SSR markers, randomly divided over the chromosomes
- Jaccard similarity:

## MODEL 2: Combination Morph/Mol. distances



## Improved DUS system POTATO



DUS test based on morphology *and* DNA:

- **First year:**
  - start of season: lightsprouttest (photo) and DNA profile
  - Growing season: morphological observations
- **End first year:**
  - DUS decision based on morphological distance and genetic distance (distinct plus thresholds)
- **Second year test for candidate varieties only when:**
  - Candidate/reference pair below 'distinct plus' threshold for morphological distance (cityblock distance  $< 0,1$ )
  - Candidate/reference pair below 'distinct plus' threshold for genetic distance (Jaccard  $< 0,2$ ).

## MODEL 2: Combination Morph/Mol. distances



### Advantages :

- A huge collection of varieties in *common knowledge* in a DNA database, not a living collection.
- Increased reliability for a candidate variety to be (or not to be) distinct from all that is known.
- Possible exclusion or inclusion reference varieties based on their DNA profiles.
- In some cases the duration of DUS trial can be reduced (cost reduction).
- Spin-off: use DNA database for other purposes than DUS.

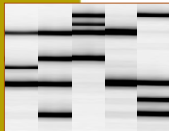


## Currently under study



### Molecular Characteristics as Predictors of 'Traditional' Characteristics

- Markers linked to disease resistance genes in tomato
- Markers for Bremia resistance in lettuce
- Markers for CMS in cabbage



### Calibration of threshold levels for the management of reference collections

- Potato
- Lettuce (in cooperation with France)
- Phalaenopsis (in cooperation with Taiwan)

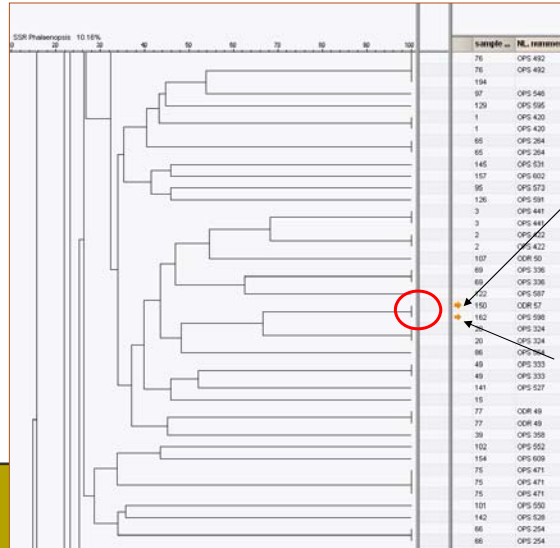




## DUS Support with DNA at Naktuinbouw

### Example 2

Compare two applications to each other and the database



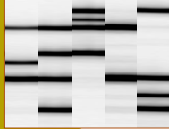
Identical profiles  
*Doritaenopsis*

and

*Phalaenopsis*  
application  
???

## Genetic Analyses - Infringements of PVP (Variety Tracer)

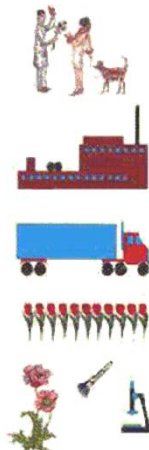
## Variety Tracer



The 'Sherlock Holmes' concept in infringement matters



## Variety identification (quality control)



Consumer

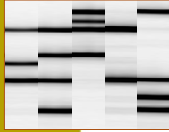
Processing

Trading

Propagation/tissue culture

Breeding and PVP

## Variety Identification



Identity questions on varietal trueness / purity

- Interchagements
- Unintentional swaps
- Variety mix-ups
- When morphological tests are not possible
- Gain time
- Aberrant plants
- Parental lines (unwanted pollination in hybrids)

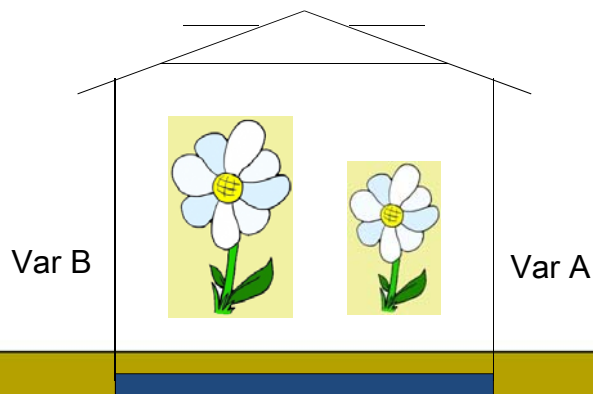
Claims:

- Suspicion of infringements of PBR/PVP
- Suspicion of repeated cropping
- Essentially derived Varieties (EDVs)

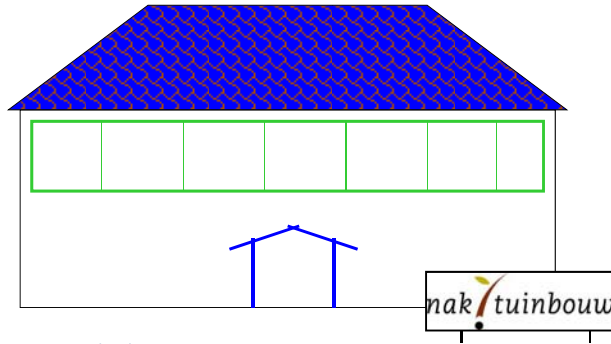
## How does Variety Tracer work...

Var A = Var B

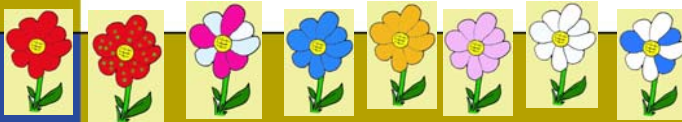
Var A  $\neq$  Var B



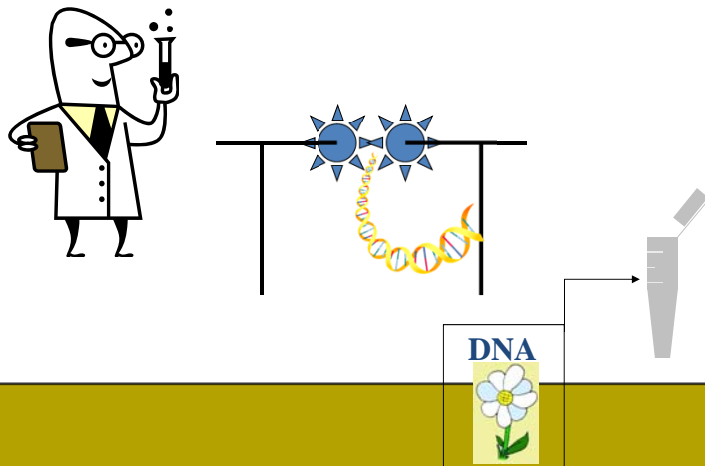
# Naktuinbouw Variety Tracer



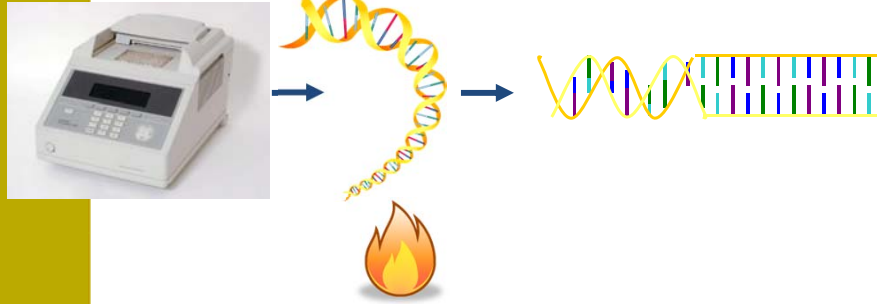
Collect reference varieties...



## In the lab...

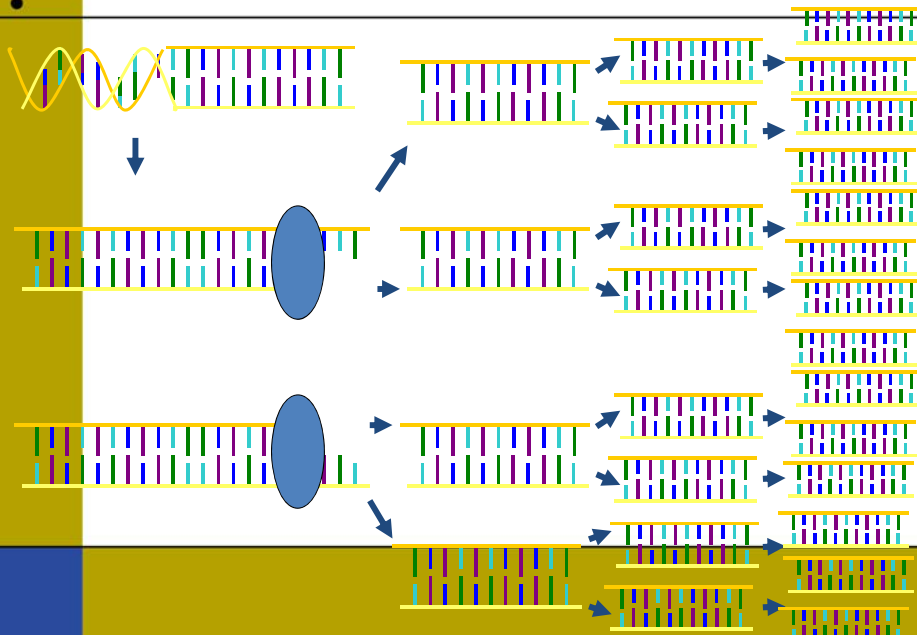


## DNA profiling...



Based on PCR technology

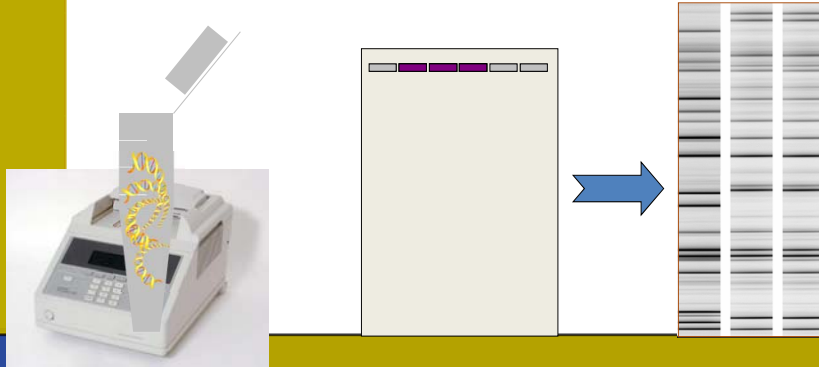
## DNA multiplication...



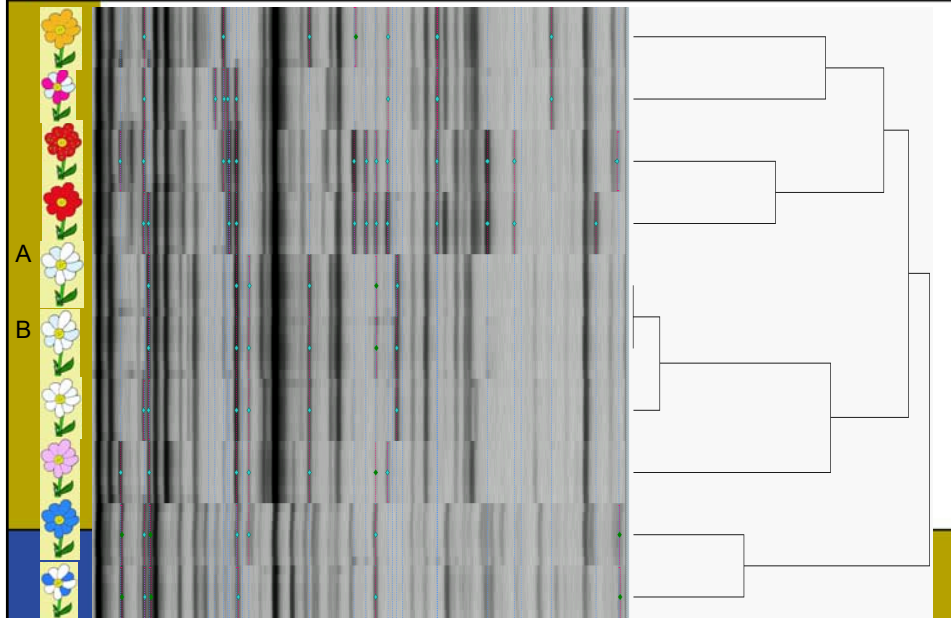
## DNA visualization...

After selective multiplication of DNA, visualization on gel.

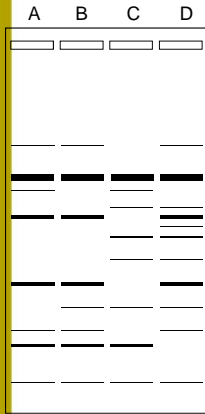
DNA fragments can be separated based on size. This results in the bar-code DNA profile



## Genetic relationships...



## From gel to data matrix



	1	2	3	4	5	6	7	8	9	10	11	12	13
A	1	1	1	0	1	0	0	0	1	0	1	1	1
B	1	1	0	0	1	0	0	0	1	1	1	1	1
C	0	1	1	1	0	0	1	1	0	1	0	1	1
D	1	1	0	1	1	1	1	1	1	1	0	1	1

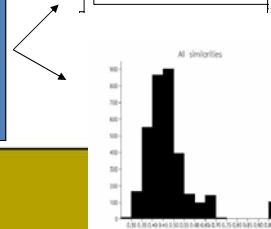
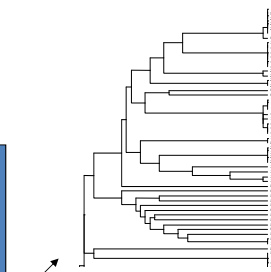
## from matrix to dendrogram

	1	2	3	4	5	6	7	8	9	10	11	12	13
A	1	1	1	0	1	0	0	0	1	0	1	1	1
B	1	1	0	0	1	0	0	0	1	1	1	1	1
C	0	1	1	1	0	0	1	1	0	1	0	1	1
D	1	1	0	1	1	1	1	1	1	1	0	1	1



	2	13	1	12	10	3	5	9	4	6	7	8	11
2	100.00												
13	41.67	100.00											
1	34.61	26.09	100.00										
12	34.61	26.09	100.00	100.00									
10	25.00	21.74	52.63	52.63	100.00								
3	30.00	24.00	61.91	61.91	55.00	100.00							
5	34.37	25.93	60.87	60.87	54.55	81.82	100.00						
9	35.71	25.00	54.55	54.55	47.62	80.00	77.27	100.00					
4	38.71	25.93	58.33	58.33	52.17	73.91	83.33	77.27	100.00				
6	36.67	28.00	59.09	59.09	57.14	76.19	86.37	71.43	95.24	100.00			
7	28.57	27.27	57.89	57.89	55.55	77.78	80.00	72.22	84.21	88.23	100.00		
8	37.50	12.50	75.00	75.00	57.14	75.00	77.78	71.43	87.50	85.71	85.71	100.00	
11	34.61	19.05	70.59	70.59	50.00	66.67	78.95	70.59	88.89	82.35	81.25	100.00	100.00

similarity matrix



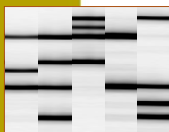




## Variety Tracer procedure



- Morphologic comparative research
  - 'Side by side' comparison
  - Identical growing conditions
  - Comparison by the relevant UPOV/CPVO protocols
  - Comparison with the original variety description
  - Availability of reference collections (DUS)



- Genetic Research
  - Choice of fingerprinting technology (AFLP/SSR/SNP/different)
  - Availability of databases
  - Experimental set-up – selection of samples

- Report,  
combination of morphological data and genetic results

## Example VT - Freesia

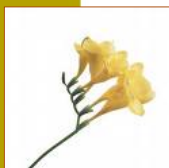


### Experimental set-up:

**Var A:** 2 samples of different sources  
1 identity sample (application sample) from -80



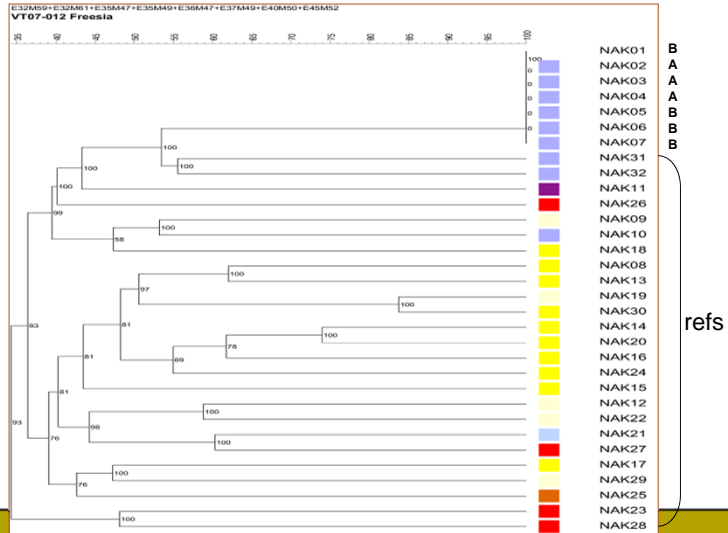
**Var B:** 1 sample from sequestered material  
2 samples different sources  
1 identity sample (application sample) from -80



**References:** 25 different varieties partially from DUS trial and partially from trade



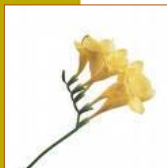
## Example VT - Freesia



clustering analysis – Jaccard

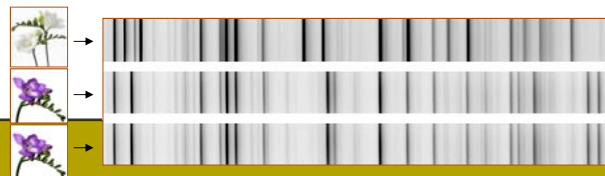
Flower color

## Example VT - Freesia



### Conclusions

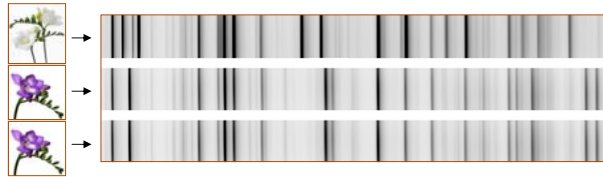
- Genetic variation in total dataset is high
- The DNA profiles of all samples of variety A and variety B were identical (100%) under the conditions used
- These results can be explained by either:
  - Variety A and B are identical, or
  - Variety B is essentially derived from Variety A



## Example VT - Freesia



### Power of Variety Tracer



Combination of morphological comparison  
and DNA analysis

**SYNERGY**

*Quality in Horticulture*