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INTERNATIONAL UNION FOR THE PROTECTION OF NEW VARIETIES OF PLANTS

Geneva

TECHNICAL WORKING PARTY FOR AGRICULTURAL CROPS

Forty-Third Session
Mar del Plata, Argentina, November 17 to 21, 2014

ADDENDUM TO DOCUMENT TWA/43/2

MOLECULAR TECHNIQUES

Document prepared by an expert from the United Kingdom

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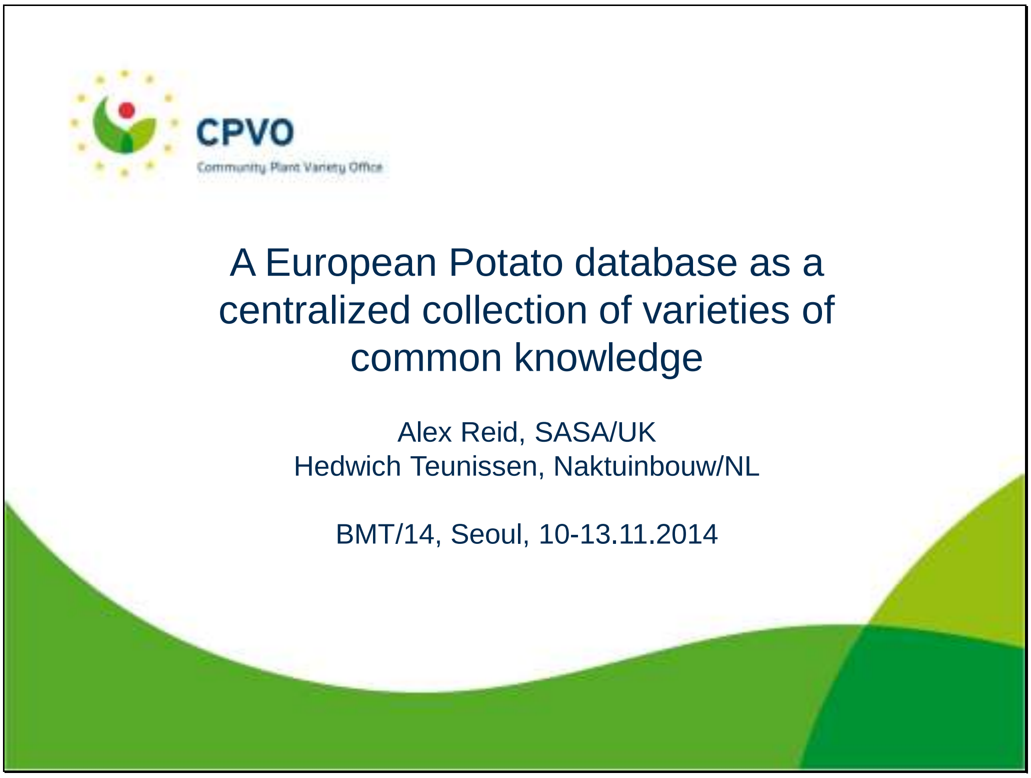
1. The annex to this document contains a presentation made by an expert from the United Kingdom by electronic means at the forty-third session of the Technical Working Party on Agricultural Crops, on “A European potato database as a centralized collection of varieties of common knowledge”.

[Annex follows]

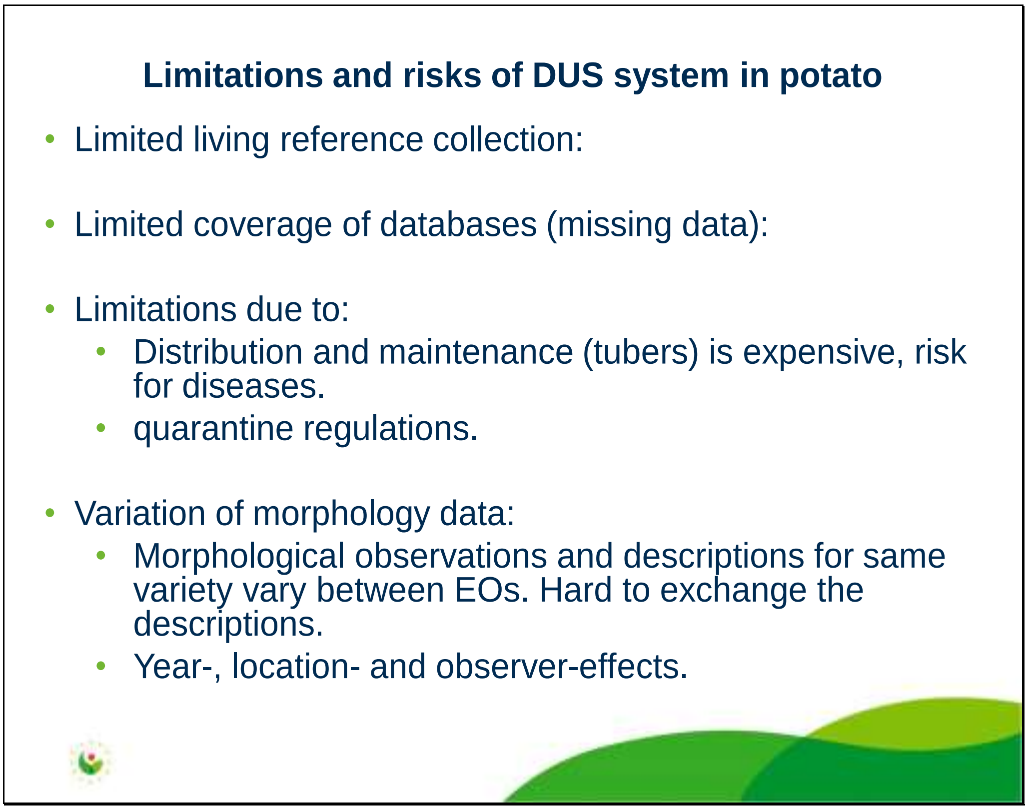


CPVO
Community Plant Variety Office

A European Potato database as a centralized collection of varieties of common knowledge

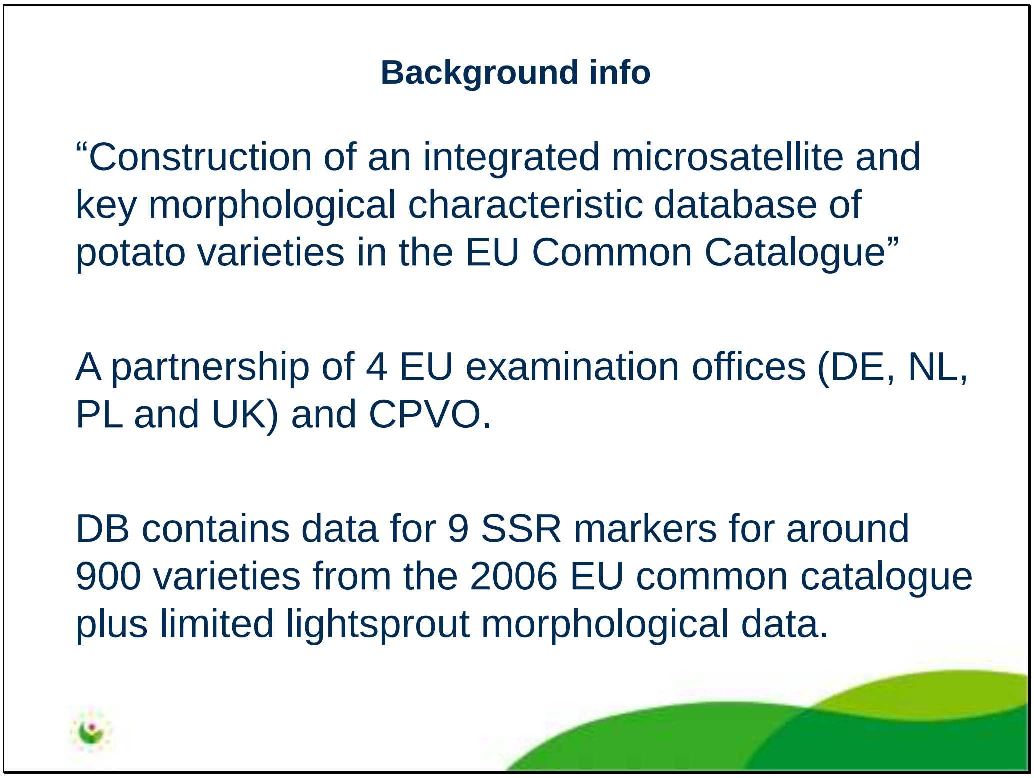
Alex Reid, SASA/UK
Hedwich Teunissen, Naktuinbouw/NL

BMT/14, Seoul, 10-13.11.2014



Limitations and risks of DUS system in potato

- Limited living reference collection:
- Limited coverage of databases (missing data):
- Limitations due to:
  - Distribution and maintenance (tubers) is expensive, risk for diseases.
  - quarantine regulations.
- Variation of morphology data:
  - Morphological observations and descriptions for same variety vary between EOs. Hard to exchange the descriptions.
  - Year-, location- and observer-effects.

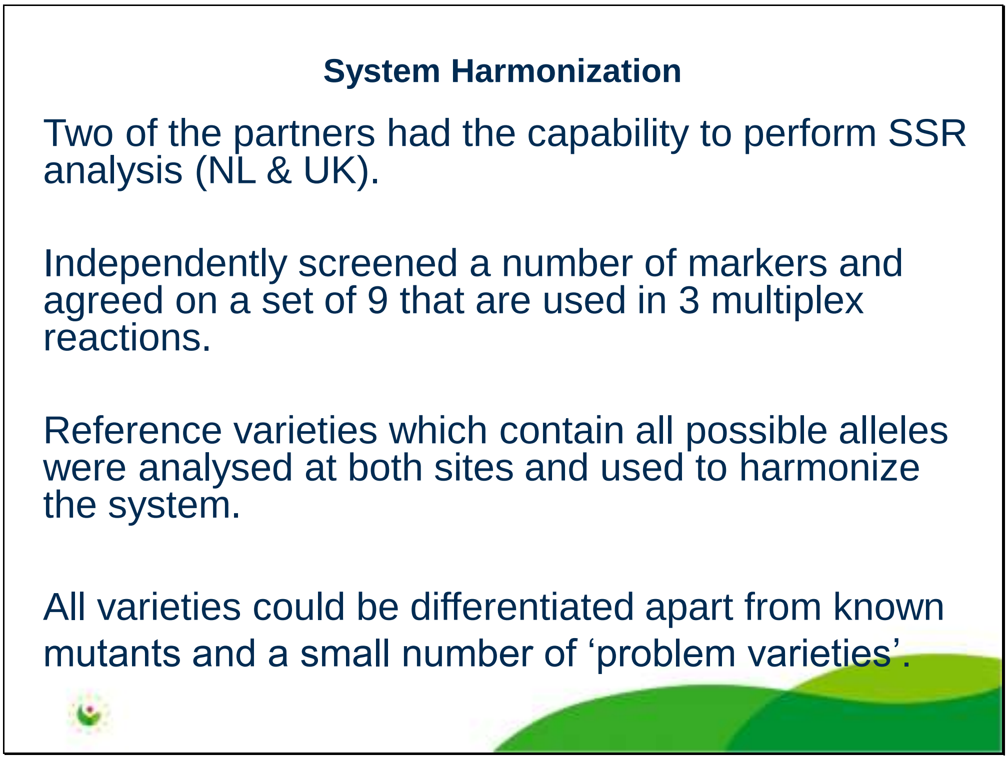


Background info

“Construction of an integrated microsatellite and key morphological characteristic database of potato varieties in the EU Common Catalogue”

A partnership of 4 EU examination offices (DE, NL, PL and UK) and CPVO.

DB contains data for 9 SSR markers for around 900 varieties from the 2006 EU common catalogue plus limited lightsprout morphological data.



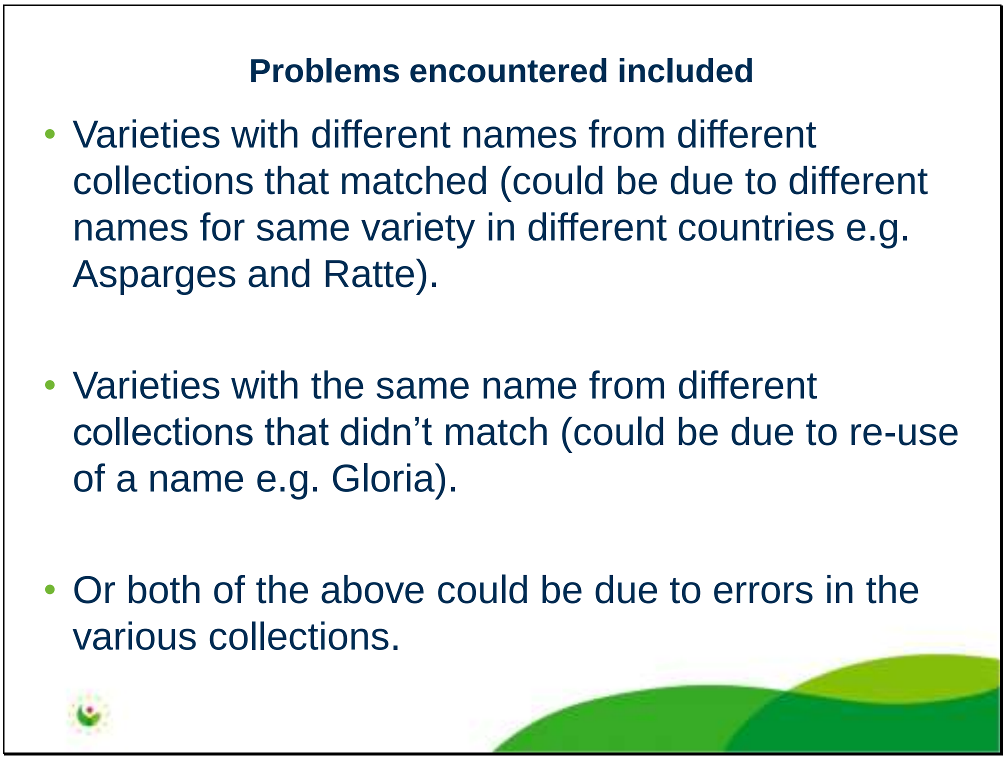
System Harmonization

Two of the partners had the capability to perform SSR analysis (NL & UK).

Independently screened a number of markers and agreed on a set of 9 that are used in 3 multiplex reactions.

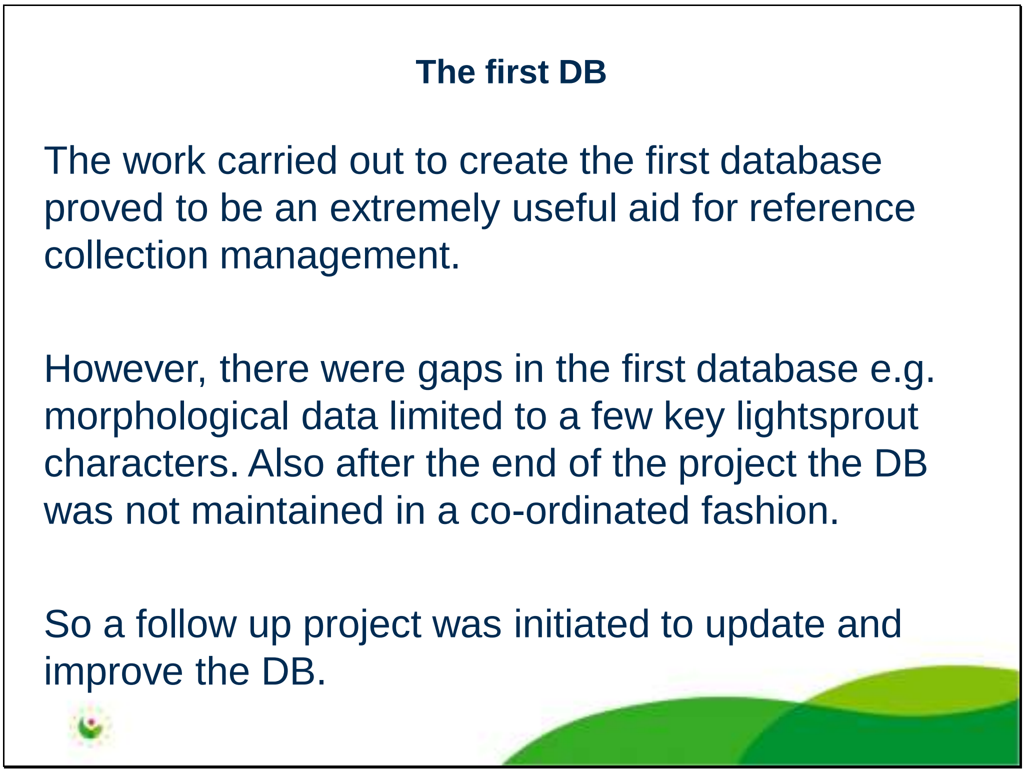
Reference varieties which contain all possible alleles were analysed at both sites and used to harmonize the system.

All varieties could be differentiated apart from known mutants and a small number of ‘problem varieties’.



Problems encountered included

- Varieties with different names from different collections that matched (could be due to different names for same variety in different countries e.g. Asparges and Ratte).
- Varieties with the same name from different collections that didn’t match (could be due to re-use of a name e.g. Gloria).
- Or both of the above could be due to errors in the various collections.



The first DB

The work carried out to create the first database proved to be an extremely useful aid for reference collection management.

However, there were gaps in the first database e.g. morphological data limited to a few key lightsprout characters. Also after the end of the project the DB was not maintained in a co-ordinated fashion.

So a follow up project was initiated to update and improve the DB.

Aim for an improved database

Improved quality of the procedure for potato DUS testing in EU.

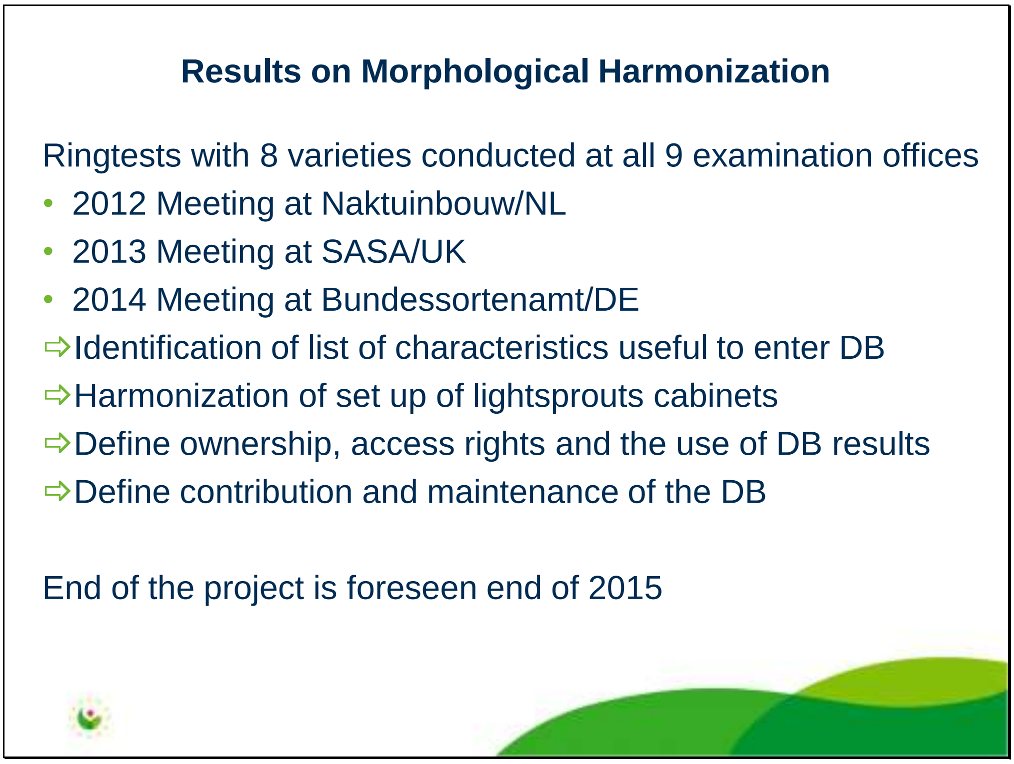
By:

- Harmonization (both morphology and markers).
- Combine morphological and molecular data.
- Improve the efficiency of DUS testing.
- Improve the management of reference collections.

Aim

Who is involved:

- Harmonization exercises for morphological data and light sprout pictures for all responsible EOs in EU
  - CPVO and 9 EU EOs: (Naktuinbouw (NL), SASA (UK), BSA (DE), COBORU (PL), OEVV (ES), DAF (IE), AGES (AT), UKZUZ (CZ), UKSUP (SK))
- Harmonization of DNA data and synchronization of old profiles
  - SASA (UK) and Naktuinbouw (NL)



Results on Morphological Harmonization

Ringtests with 8 varieties conducted at all 9 examination offices

- 2012 Meeting at Naktuinbouw/NL
- 2013 Meeting at SASA/UK
- 2014 Meeting at Bundessortenamt/DE

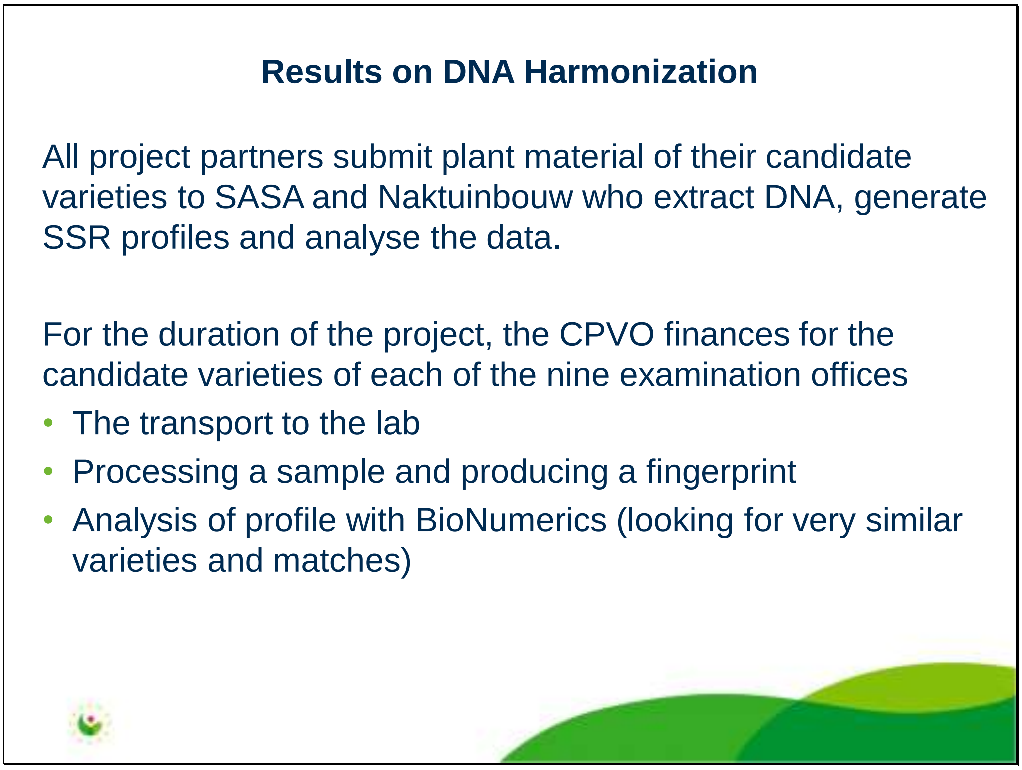
⇨Identification of list of characteristics useful to enter DB

⇨Harmonization of set up of lightsprouts cabinets

⇨Define ownership, access rights and the use of DB results

⇨Define contribution and maintenance of the DB

End of the project is foreseen end of 2015

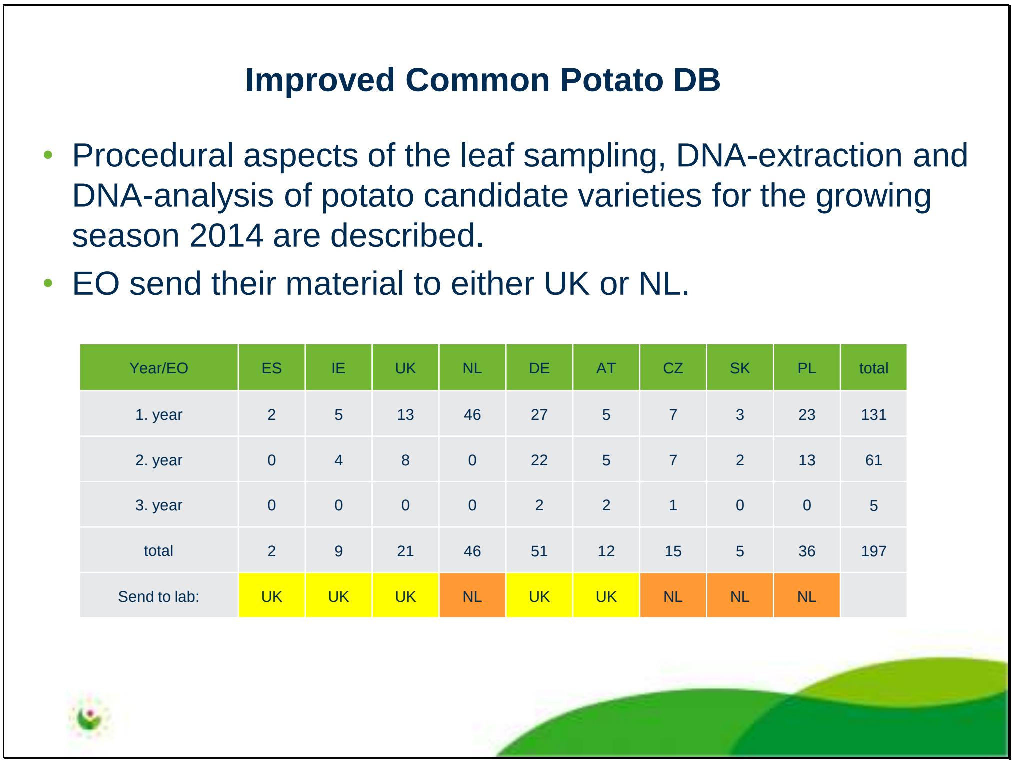


Results on DNA Harmonization

All project partners submit plant material of their candidate varieties to SASA and Naktuinbouw who extract DNA, generate SSR profiles and analyse the data.

For the duration of the project, the CPVO finances for the candidate varieties of each of the nine examination offices

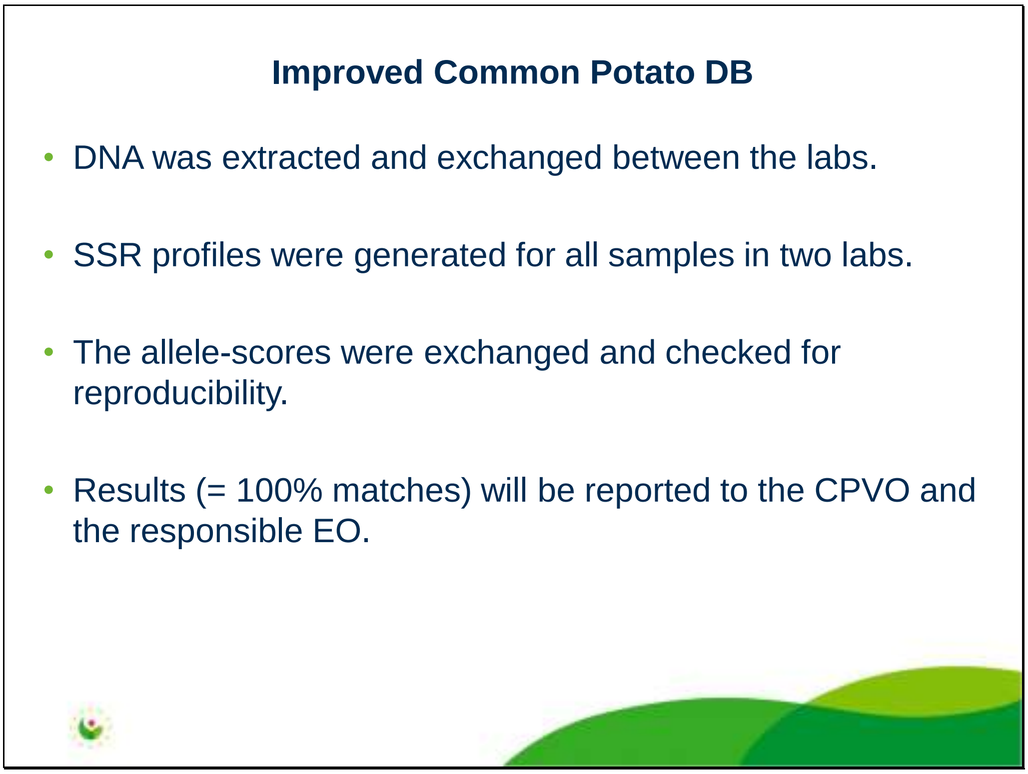
- The transport to the lab
- Processing a sample and producing a fingerprint
- Analysis of profile with BioNumerics (looking for very similar varieties and matches)



Improved Common Potato DB

- Procedural aspects of the leaf sampling, DNA-extraction and DNA-analysis of potato candidate varieties for the growing season 2014 are described.
- EO send their material to either UK or NL.

Year/EO	ES	IE	UK	NL	DE	AT	CZ	SK	PL	total
1. year	2	5	13	46	27	5	7	3	23	131
2. year	0	4	8	0	22	5	7	2	13	61
3. year	0	0	0	0	2	2	1	0	0	5
total	2	9	21	46	51	12	15	5	36	197
Send to lab:	UK	UK	UK	NL	UK	UK	NL	NL	NL	

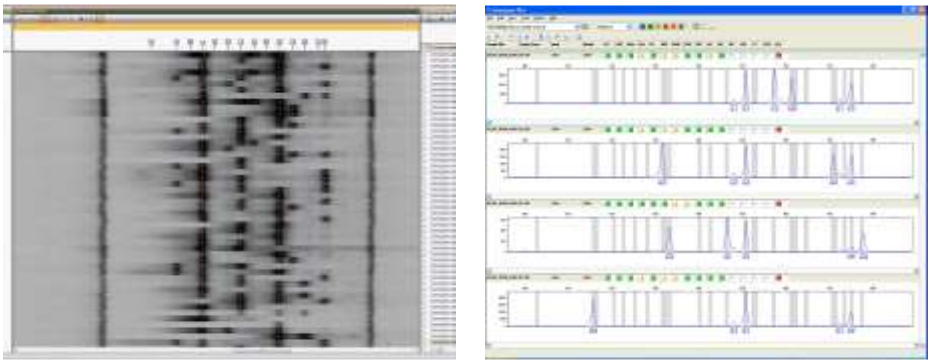


Improved Common Potato DB

- DNA was extracted and exchanged between the labs.
- SSR profiles were generated for all samples in two labs.
- The allele-scores were exchanged and checked for reproducibility.
- Results (= 100% matches) will be reported to the CPVO and the responsible EO.

Results of SSR analysis

- Reproducibility



- **Within** each lab reproducibility is high (100%)
- Reproducibility **between** labs is more challenging due to different platforms used

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Results of SSR analysis

- Reproducibility

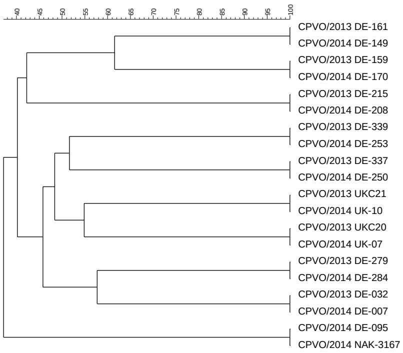
Sample	SSR1 NL	SSR1 UK	0019 NL	0019 UK	2005 NL	2005 UK	2028 NL	2028 UK	3009 NL	3009 UK	3012 NL	3012 UK	3023 NL	3023 UK	5136 NL	5136 UK	5148 NL	5148 UK
3460	BDFI	BDFI	BDF	BDF	BD	BD	ACDE	ACDE	**G**	**DG**	CF	CF	ABD	ABD	CEF	CEF	GJMP	GJMP
3461	DF	DF	BDG	BDG	ABD	ABD	ABC	ABC	G	G	BCF	BCF	ABD	ABD	FH	FH	BMO	BMO
3462	DIJ	DIJ	BD	BDE	ABD	ABD	ABC	ABC	FG	FG	BC	BC	BD	BD	CEF	CEF	JMO	JMO
3463	DI	DI	BFH	BFH	ABD	ABD	ABCE	ABCE	G	G	BCF	BCF	ABD	ABD	**[D?]EF**	**DEF**	CJOP	CJOP
3464	DF	DF	BDH	BDH	AB	AB	ABCE	ABCE	G	G	BC	BC	D	D	CFH	CFH	GJO	GJO
3465	DEI	DEI	BFH	BFH	ABD	ABD	ABCE	ABCE	G	G	BCF	BCF	AD	AD	CEFH	CEFH	JOW	JOW
3466	BDIN	BDIN	EF	EF	ABC	ABC	A	A	DG	DG	BC	BC	AD	AD	**C[D?]F**	**CDF**	FIP	FIP
3467	FI	FI	F	F	ABD	ABD	AE	AE	BG	BG	BC	BC	A	A	FH	FH	BJOP	BJOP
3468	DFIL	DFIL	BF	BF	AD	AD	ACE	ACE	BFG	BFG	BC	BC	AC	AC	F	F	BE	BE
3469	DF	DF	DEG	DEG	ABD	ABD	ABCE	ABCE	FGL	FGL	BD	BD	AD	AD	CFH	CFH	IJO	IJO
3470	DI	DI	BDF	BDF	AB	AB	BCI	BCI	FK	FK	BCD	BCD	ABD	ABD	CFH	CFH	BFJ	BFJ
3471	BDI	BDI	NULL	NULL	ABD	ABD	ABC	ABC	BFG	BFG	ABD	ABD	D	D	CFH	CFH	GIJP	GIJP
3472	BDFI	BDFI	EF	EF	BD	BD	ACD	ACD	BFG	BFG	BCD	BCD	AD	AD	CH	CH	AGIJ	AGIJ
3473	DFL	DFL	F	F	BDF	BDF	AD	AD	FG	FG	BD	BD	AD	AD	CEFH	CEFH	BGOP	BGOP
3474	ADF	ADF	F	F	BD	BD	BD	BD	FG	FG	ABC	ABC	AD	AD	CFH	CFH	BJOW	BJO
3475	ABDI	ABDI	BDE	BDE	BD	BD	ACD	ACD	G	G	BDF	BDF	D	D	CF	CF	AIJP	AIJP

Two types of problems:
1. One lab calls a definite allele (present or absent) - 🟩
the other calls it as questionable (actually not a real problem).

2. Both labs have different calls (a real problem). 🟥

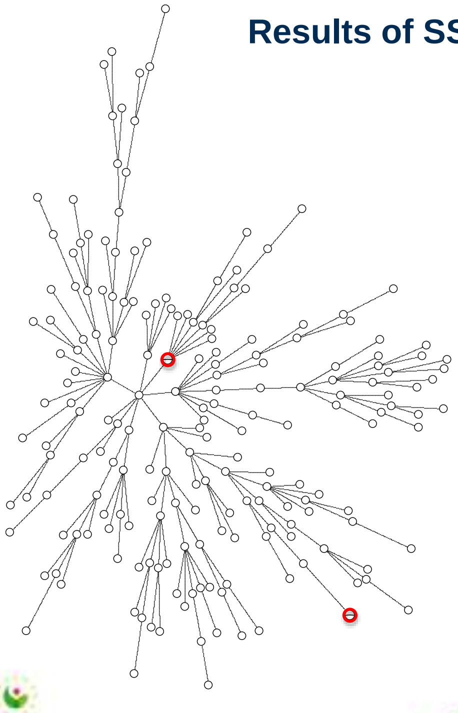
Not *that* big an issue as differences are generally from a limited set of alleles that we already know can be a problem or alleles that are called as questionable by one of the labs. (We made scoring rules to overcome this minor problem).

Results of SSR analysis

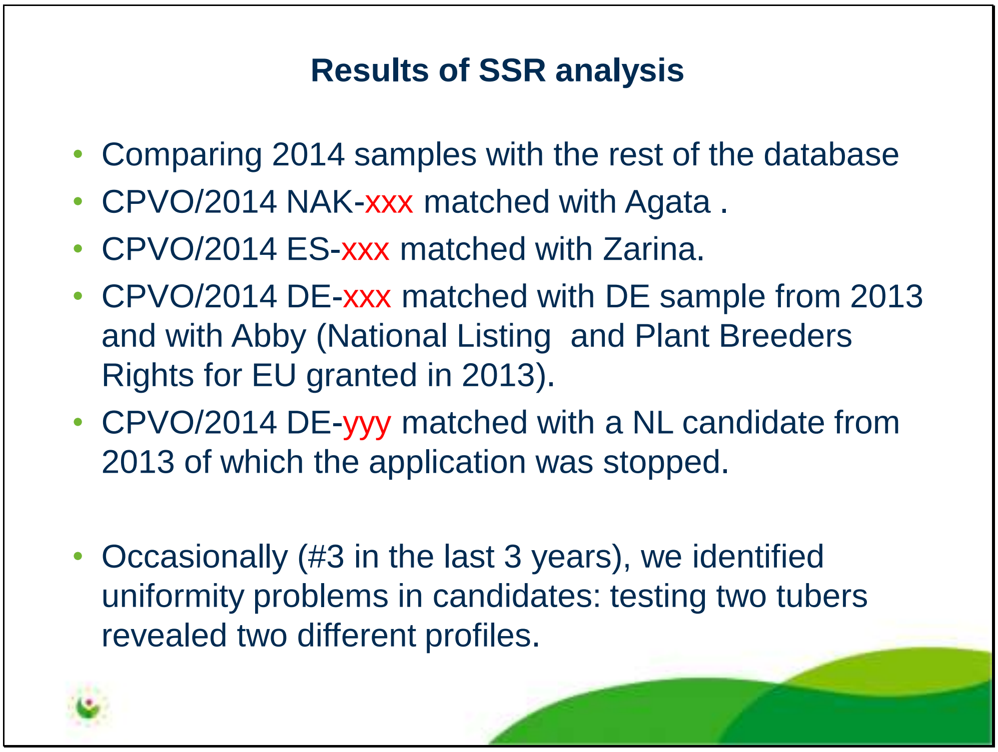


- Reproducibility of 2013 and 2014 samples was good

Results of SSR analysis



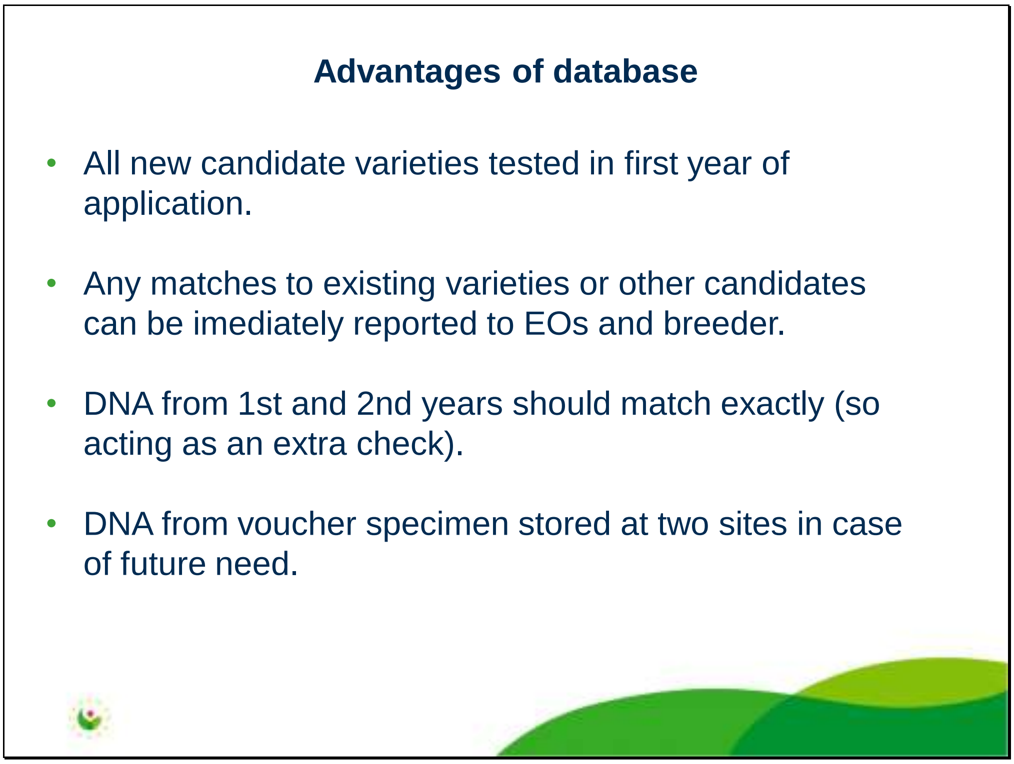
- Tree for 2014 samples shows all candidates are unique apart from 2 pairs of samples.
- one from NL the other from DE both 1st year applications.
- One from NL matched Agata from PL.



Results of SSR analysis

- Comparing 2014 samples with the rest of the database
- CPVO/2014 NAK-xxx matched with Agata .
- CPVO/2014 ES-xxx matched with Zarina.
- CPVO/2014 DE-xxx matched with DE sample from 2013 and with Abby (National Listing and Plant Breeders Rights for EU granted in 2013).
- CPVO/2014 DE-yyy matched with a NL candidate from 2013 of which the application was stopped.

- Occasionally (#3 in the last 3 years), we identified uniformity problems in candidates: testing two tubers revealed two different profiles.



Advantages of database

- All new candidate varieties tested in first year of application.

- Any matches to existing varieties or other candidates can be imediately reported to EOs and breeder.

- DNA from 1st and 2nd years should match exactly (so acting as an extra check).

- DNA from voucher specimen stored at two sites in case of future need.

Future

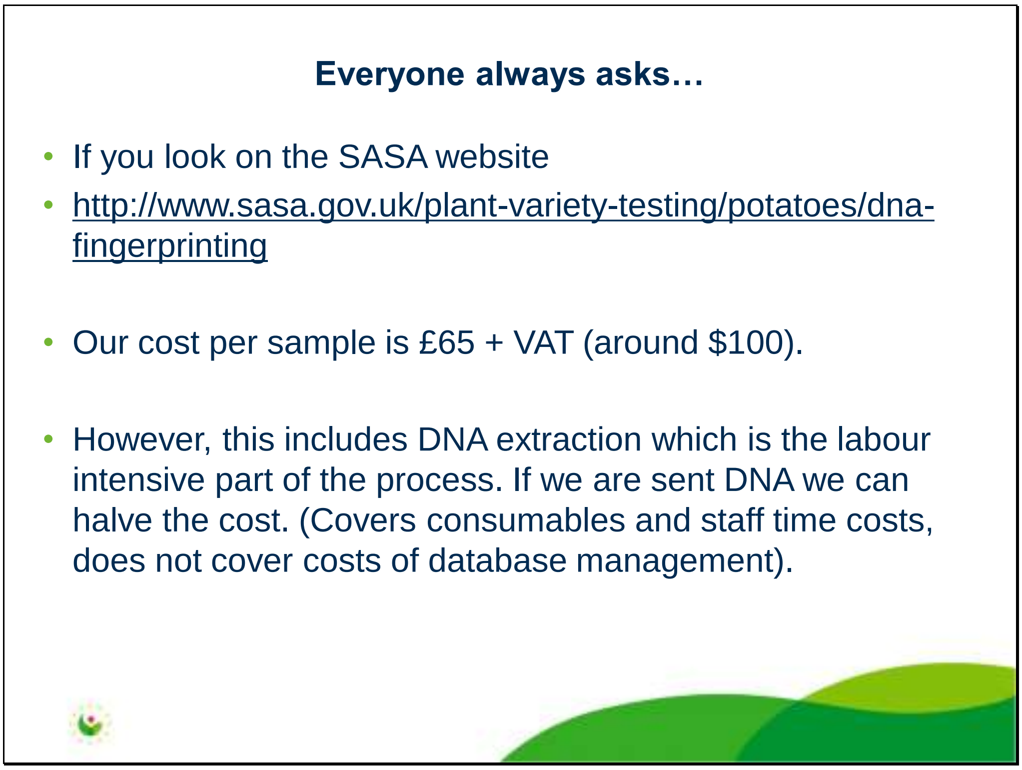
- To keep the DB up to date we need information back from EOs concerning
  - Successful applications and subsequent **names**
  - Withdrawn samples
- Further investigation into how the combination of SSR and molecular characteristics could work.
- Platform to share data with EOs and CPVO if desired (NL and UK already share SSR data).

Proposal

- We are using this system in Europe as an aid to DUS testing and reference collection maintenance.

- The usefulness of the database increases with the number of samples it contains.

- A suggestion was made at the BMT that it would be extremely valuable to extend this work to include varieties from the rest of the world. (Canada already use the same system to monitor their collection and found it relatively simple to harmonize with us).



Everyone always asks…

- If you look on the SASA website
- http://www.sasa.gov.uk/plant-variety-testing/potatoes/dna-fingerprinting

- Our cost per sample is £65 + VAT (around $100).

- However, this includes DNA extraction which is the labour intensive part of the process. If we are sent DNA we can halve the cost. (Covers consumables and staff time costs, does not cover costs of database management).

[End of Annex and of document]