

SASA



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

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Limitations and risks of DUS system in potato

“The trial for DUS testing consists of new varieties in tests (hereinafter: candidate varieties) and varieties of reference collections. Candidate varieties are compared with varieties of reference collections for the purpose of determination of distinctness.”

Limitations and risks of DUS system in potato

Limited living reference collections:

- Distribution and maintenance of tubers is expensive.

- Risk for spreading diseases hence quarantine regulations.

Variation of morphological data:

- Morphological observations and descriptions for same variety vary between EOs. Hard to exchange the descriptions.

- Year, location and observer effects.

Background information

“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 GB) and CPVO.

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

SSR harmonization

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

Independently screened a number of markers and agreed on a set of 9 that are used in 3 multiplex reactions. These 9 markers currently yield a total of 113 possible alleles.

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'.

SASA database currently contains over 2000 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. Asparagus 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 either in collection maintenance (variety mix ups) or the non-inclusion of a crucial reference variety.

Errors we know about!

The European Cultivated Potato Database

HIGHER TAXON: Solanaceae
GENUS: Solanum L.
Solanum tuberosum L. cv. Royal Kidney
Pedigree:
Breeder: 9 [9]
Findlay, A., Auchtermuchty, Scotland [5,13]
Breeder's rights: No [13]
Synonyms:
National list: 1890 [20]
1899 [2,9]
1977 [13]

RK national listed in 1890

The European Cultivated Potato Database

HIGHER TAXON: Solanaceae
GENUS: Solanum L.
Solanum tuberosum L. cv. King David
Pedigree: Nicola x Dr McIntosh [13]
Breeder: Dr Avi Nachmas, Pitada Food Industries Ltd, Kefar, Shinarayahu, Israel [13]
Breeder's rights: Yes [13]
Synonyms:
National list: 2002 [13]

KD national listed in 2002

The first database

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 database was not maintained in a co-ordinated fashion.

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

Aim for an improved database

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

By:

Harmonization (both morphology and markers).

Combining morphological and molecular data.

Leading to:

Improved efficiency of DUS testing.

Improved management of reference collections.

The new database

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 (GB), BSA (DE), COBORU (PL), OEVV (ES), DAF (IE), AGES (AT), UKZUZ (CZ), UKSUP (SK))

Harmonization of DNA data and synchronization of old profiles

SASA (GB) and Naktuinbouw (NL)

Morphological harmonization

Ringtests with 8 varieties conducted at all 9 examination offices

2012 Meeting at Naktuinbouw/NL

2013 Meeting at SASA/ GB

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 by the end of 2015

Improved common potato database

In 2014 EOs sent their material to either GB or NL.

Samples were submitted as leaf material but this proved to have difficulties.

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 database

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) were reported to the CPVO and the responsible EO.

Results of SSR analysis

Reproducibility

Sample	SSR1 NL	SSR1 GB	0019 NL	0019 GB	2005 NL	2005 GB	2028 NL	2028 GB	3009 NL	3009 GB	3012 NL	3012 GB	3023 NL	3023 GB	5136 NL	5136 GB	5148 NL	5148 GB
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:

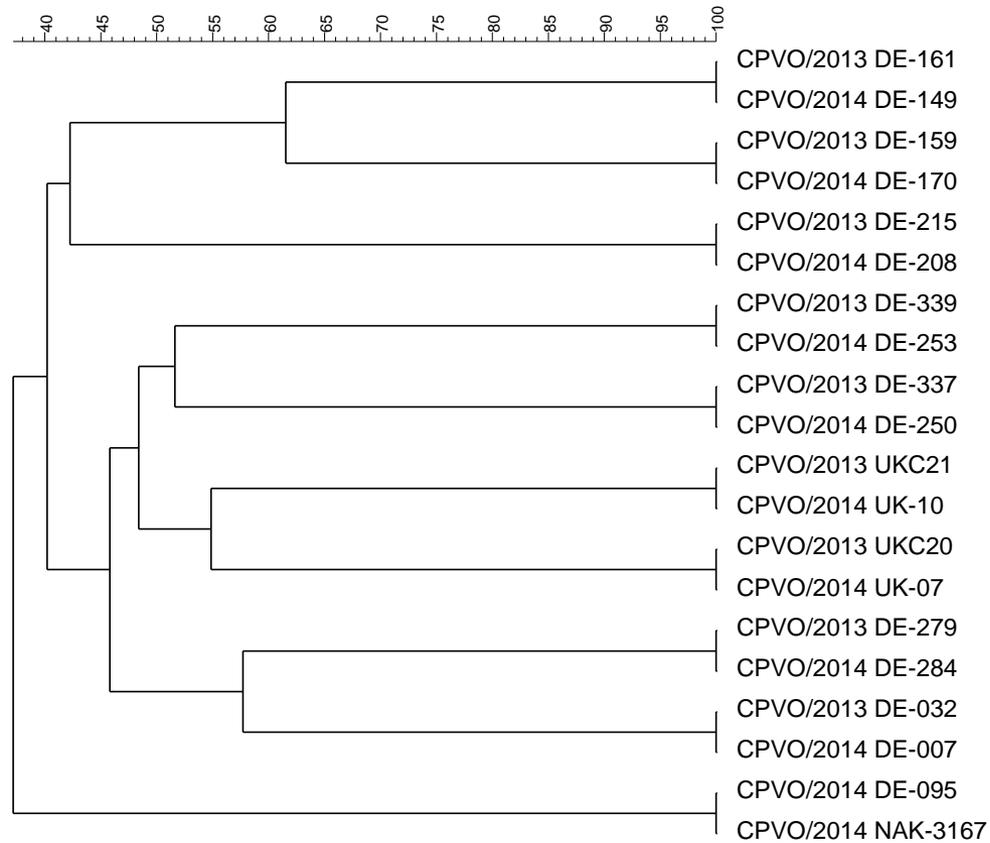
1. One lab calls a definite allele (present or absent) - 
the other calls it as questionable (actually not a real problem).

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).

Reproducibility

Comparing 2013 and 2014 samples



N.B. Samples are analysed blind

2014 results

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 times in the last 3 years), we identified uniformity problems in candidates: testing two samples revealed two different profiles.

DNA fingerprinting protocol 2015

All project partners submit 2 tubers of their candidate varieties to SASA where the DNA is extracted. (DNA for Dutch varieties extracted by Naktuinbouw.)

SSR profiles from tuber A generated at SASA

SSR profiles from tuber B generated at Naktuinbouw

Results from A and B tubers analysed together using BioNumerics software.

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

Advantages of database

All new candidate varieties tested in first year of application.

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

DNA from 1st and 2nd years should match exactly (so acting as an extra check). N.B. We are considering if this is necessary.

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

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 2014 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 and are keen to be involved.