

DNA fingerprinting of potato varieties at SASA

Alex Reid

Need for a genotyping system



Culture collection ca. 1300 varieties



Nuclear stock unit
ca. 1000 varieties

Development of a genotyping system

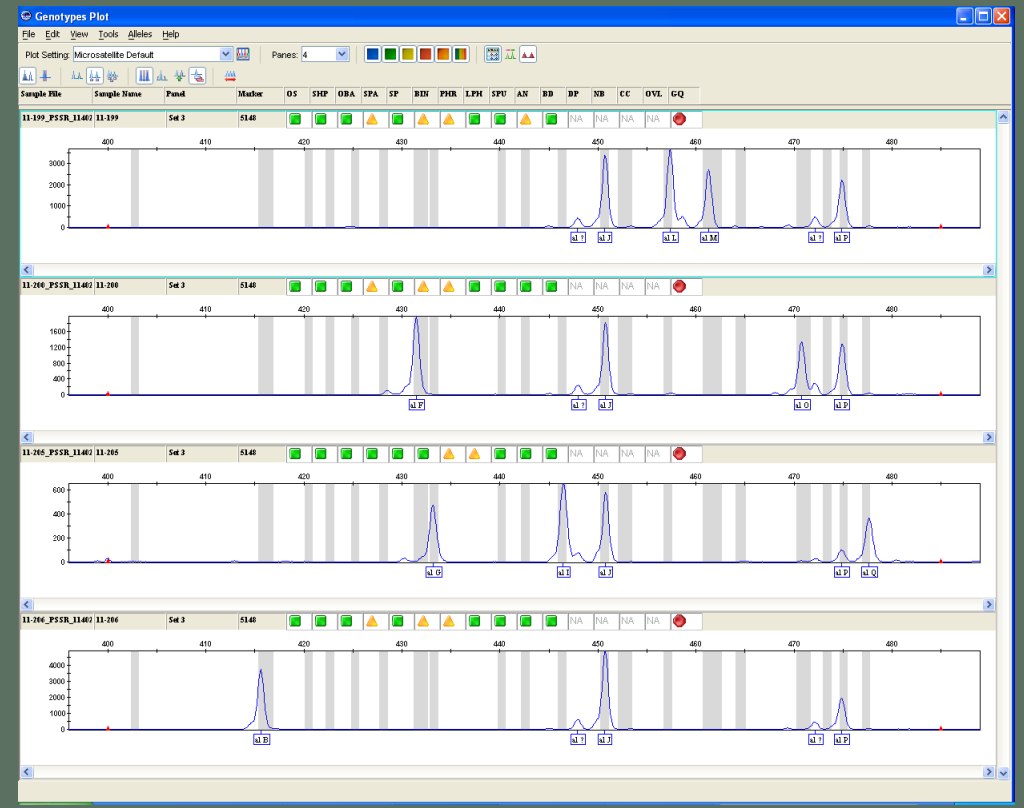
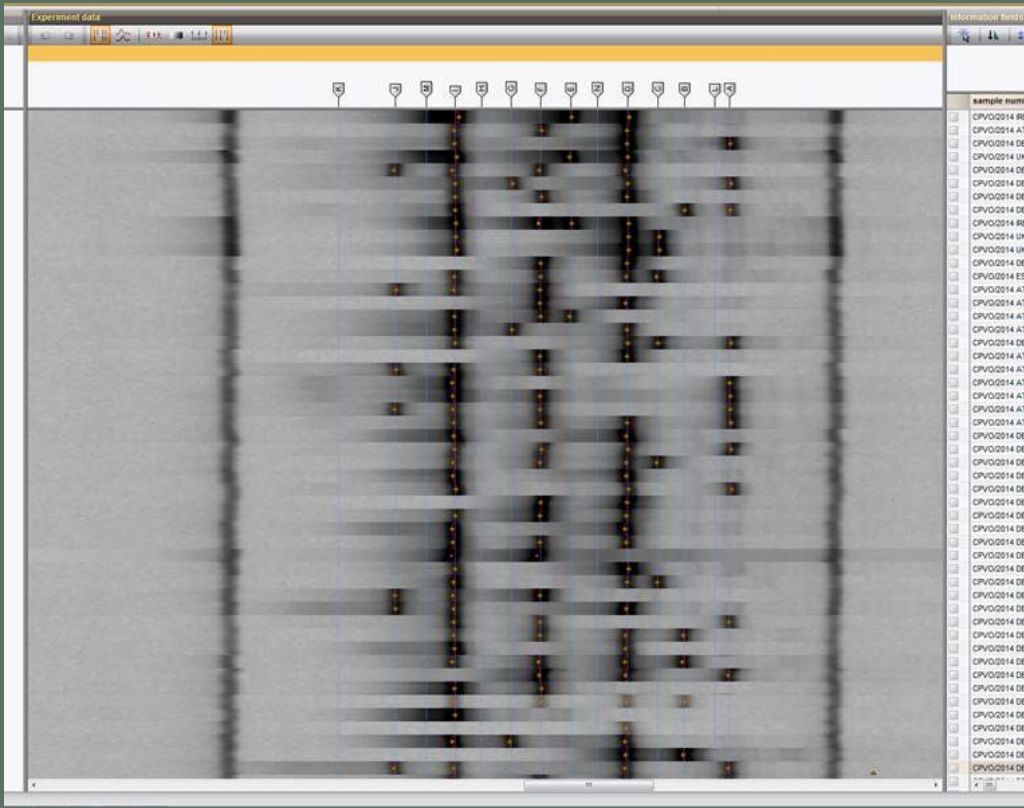
The current system began in 2003 when Lysbeth Hof and I decided to pull our resources and work on a genotyping system for potato.

By 2004 we had a set of 9 markers that were robust, easy to interpret and could be multiplexed. This is the method still in use for DUS testing in Europe.

At SASA we also use an additional 3 markers in a fourth multiplex.

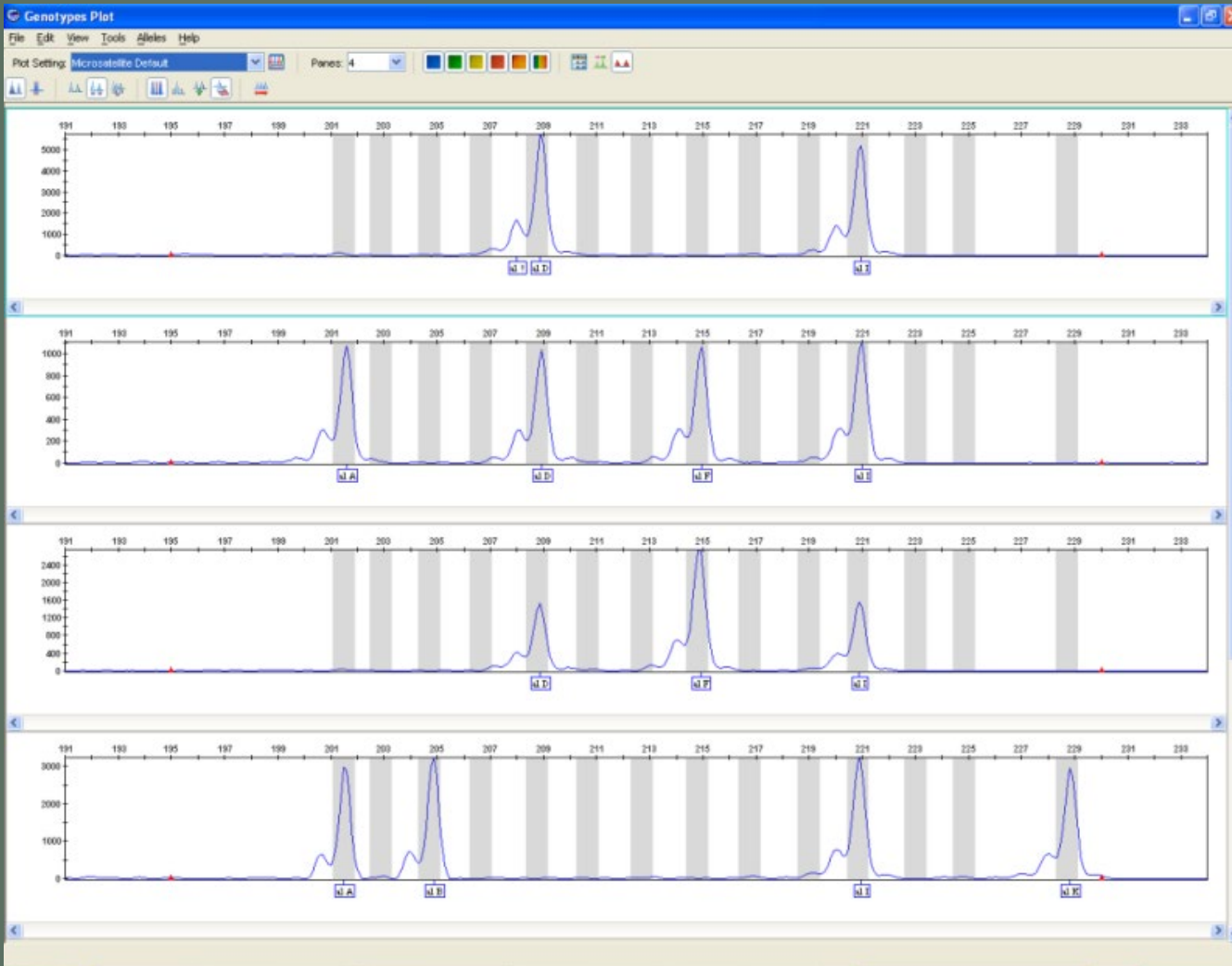
Marker	Repeat	LG	# Alleles	PIC
0019	(AT) ₇ (GT) ₁₀ (AT) ₄ (GT) ₅ (GC) ₄ (GT) ₄	VI	18	0.92
3009	(TC) ₁₃	VII	18	0.8
SSR1	(TCAC) _n	VIII	15	0.93
2005	(CTGTTG) ₃	XI	7	0.8
3012	(CT) ₄ .(CT) ₈	IX	10	0.87
3023	(GA) ₉ .(GA) ₈ .(GA) ₄	IV	6	0.79
2028	(TAC) ₅ .(TA) ₃ .(CAT) ₃	XII	13	0.9
5136	(AGA) ₅	I	12	0.92
5148	(GAA) ₁₇	V	24	0.98
1016	(TCT) ₉	VII	17	
1024	(TTG) ₆	VIII	8	
2022	(CAA) ₃ .(CAA) ₃	II	7	

SASA and Naktuinbouw use different platforms



Within each lab reproducibility is high (100%).
Reproducibility between labs is more challenging as size calling of bands/peaks can be different due to hardware differences. Therefore, we harmonized the two systems and call alleles by letters

Scoring alleles

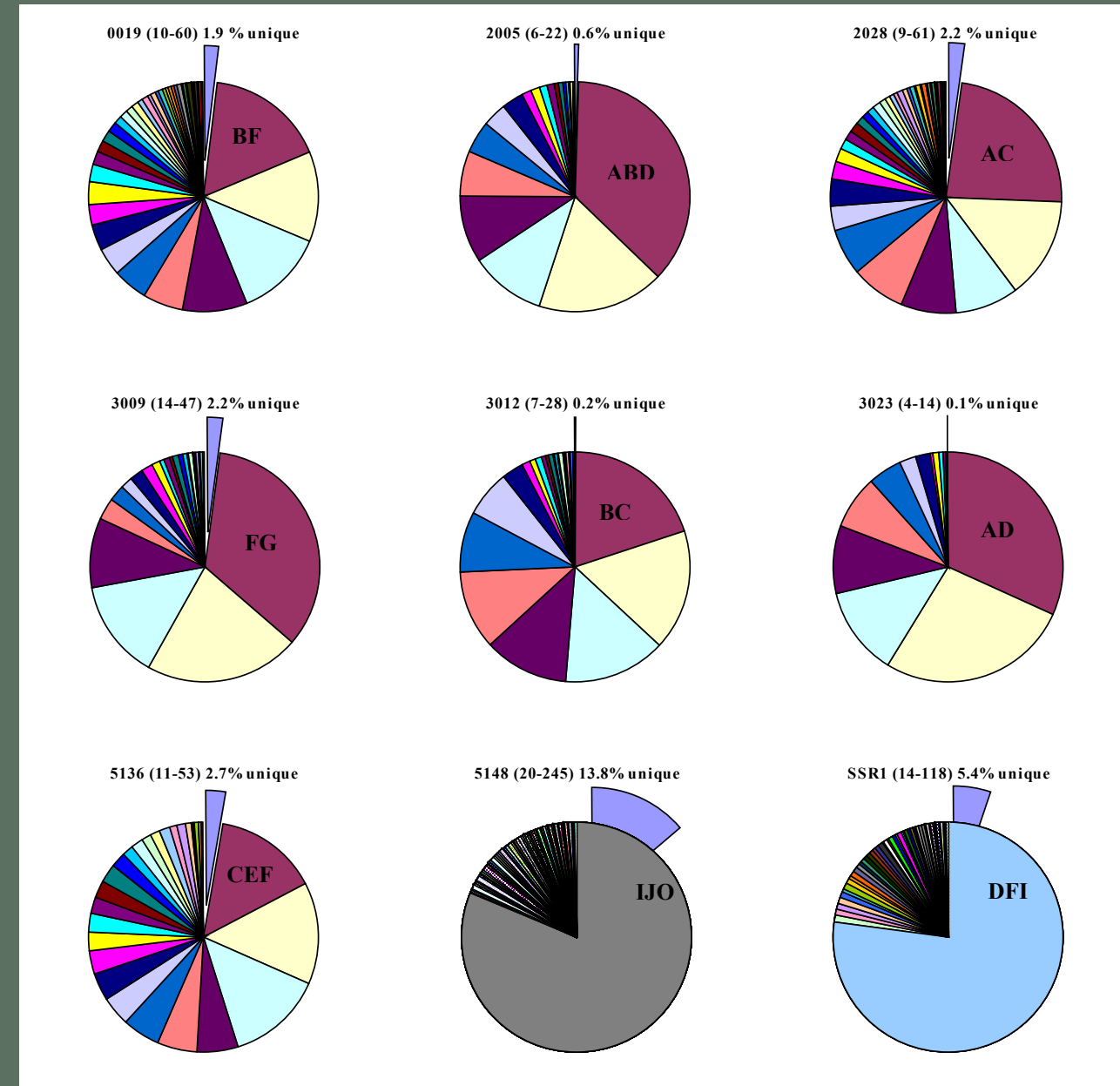


Sample	A	B	C	D	E	F	G	H	I	J	K	L	M
1	0	0	0	1	0	0	0	0	1	0	0	0	0
2	1	0	0	1	0	1	0	0	1	0	0	0	0
3	0	0	0	1	0	1	0	0	1	0	0	0	0
4	1	1	0	0	0	0	0	0	1	0	1	0	0

Marker discriminative power

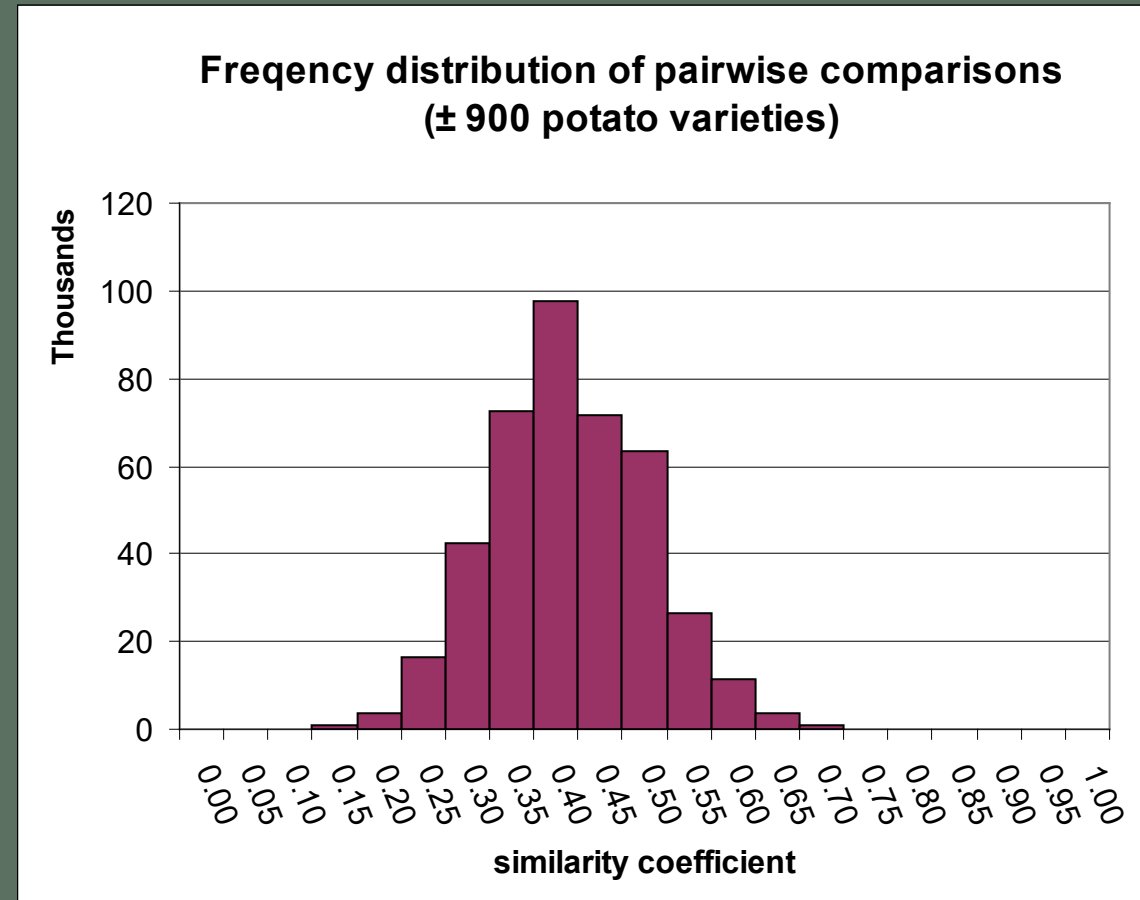
Name	# alleles 2006	# alleles 2024
STMS 0019	10	18
STMS 2005	6	7
STMS 2028	9	13
STMS 3009	14	18
STMS 3012	7	10
STMS 3023	4	6
STMS 5136	11	12
STMS 5148	20	24
STMS SSR1	14	15
Total	95	123

With the additional 3 markers in use at SASA the total number of alleles is 155



Marker discriminative power

Using the 9 markers 99.5% of all varieties could be differentiated.
Plotting pairwise comparisons show that 99.9985% of all pairs have a similarity value of less than 85%. They also differ by at least 4 alleles.



Highly similar varieties

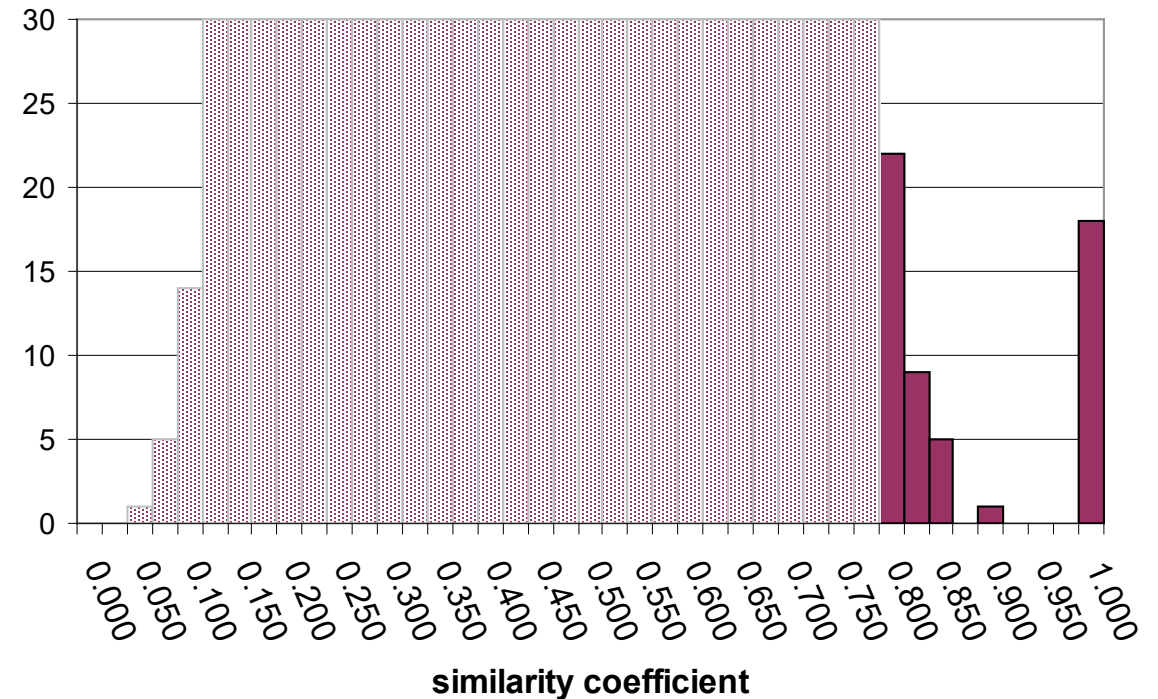
Looking at the 0.5% of varieties we couldn't differentiate.

Most are known mutants (e.g. King Edward/Red King Edward).

Some are synonyms (e.g. Asparagus/Ratte).

The rest we strongly suspect as historic mislabels.

Frequency distribution of pairwise similarities:
close-up of upper tail end



The DUS test



Two tubers of each candidate going through DUS are tested. One is profiled at SASA the other at Naktuinbouw.

Results are compared to check for any differences caused by the different electrophoresis systems and possible admixes.

The DNA profiles are checked against the entire database of varieties typed during previous projects and other varieties of 'common knowledge' (well over 3000 varieties).

Matches of 85% and above are reported to the EO that submitted the sample for further investigation.

All DNA samples are stored frozen in case they are needed for whatever reason by the breeder of the variety in the future.

Three co-funded CPVO projects



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

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

“Construction of a European Potato database with varieties of common knowledge and its implementation in the potato DUS testing system.”

For more information

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

A. Reid, L. Hof, G. Felix, B. Rücker, S. Tams, E. Milczynska, D. Esselink, G. Uenk, B. Vosman & A. Weitz

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Evaluation of Simple Sequence Repeat (SSR) Markers Established in Europe as a Method for the Identification of Potato Varieties Grown in Canada

Marie-José Côté, Lisa Leduc & Alex Reid

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Chapter 23

Potato Cultivar Genome Analysis

Alex Reid, Lysbeth Hof, Danny Esselink, and Ben Vosman

Summary

Due to the yearly increase in the numbers of new potato varieties obtaining Plant Breeders' Rights the reliable maintenance of large culture collections of reference varieties for DUS testing is becoming more and more difficult, as accidental mix ups might occur. Efficient identification methods and databases can act as an aid to overcome this problem. Identification of cultivars by morphological characteristics is a highly skilled and time-consuming task, and for these reasons a rapid and robust method for variety differentiation has become extremely desirable. By use of a set of nine microsatellite (SSR) markers we can differentiate over 1,000 cultivars, including the majority of varieties on the European Union Common Catalogue, but excluding somaclonal variants (e.g. Red King Edward and King Edward) and mutants. The whole identification process from DNA extraction to accurate identification can be carried out in a single day.

Key words: Genotyping, Microsatellite, SSR, Potato, Variety identification.

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I'm sure you are all wondering...



How long does it take and what does it cost?

It *can* be done in a single day but that is very labour intensive.

It is more efficient to batch samples in multiples of 24.

In this case it costs us around £75/sample (€87 at current exchange rate).

Thank you

If you have any further questions you can email me at

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