Final Report of the AEGIS project

Identification of old potato clones having unreliable variety names by means of fingerprinting using microsatellite (SSR) markers to assist in setting up the AEGIS collection for potato cultivars

All results can be downloaded from:
http://documents.plant.wur.nl/cgn/pgr/AEGISpotato/

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Introduction

The true identity (cultivar name) of potato clones in different collections is not always clear or correct. This is hampering the selection of the Most Appropriate Accessions (MAA's) by the countries for the AEGIS collection (www.aegis.cgiar.org).

- In particular for old potato cultivars the clone can be mislabelled, as reported by H. Campbell from SASA (Frese & Hoekstra, 2009). This was also observed during work carried out for the Community Plant Variety Office (CPVO, an agency of the European Union, located in Angers, France, administering plant breeders' rights) when typing the varieties on the EU Common Catalogue (Reid *et al*., 2011).
- Some variety names have been used more than once (e.g. Gloria 1921, 1937, 1972) and it is not always known to the curator what the true identity of the clone in their collection is.
- Based on SSR data, K. Dehmer (IPK, Germany) found for old blue/purple fleshed potato varieties that different names may be synonyms for the same clone.
  Summary provided by K. Dehmer: a set of 15 SSR markers was applied onto 26 blue fleshed accessions of the IPK Genebank. Only seven different SSR patterns/genotypes were identified. Four unique genotypes were represented by one GLKS accession each, while the other three genotypes were attributed to three duplication groups consisting of thirteen, five and four GLKS accessions, respectively.

In particular clones of presumably old potato cultivars can be mislabelled. This may be caused by incorrect information from the germplasm donor, or errors/interchanges made in following maintenance years. The classical differentiation of cultivars based on morphological characteristics is a highly skilled and time-consuming task.
To assist in granting Plant Breeders' Rights for new potato varieties, a standard fingerprinting method has been developed (Reid *et al*. 2009 and 2011). It is a rapid and robust method for variety differentiation using nine microsatellite (SSR) markers. Over 6,000 clones (cultivars) have successfully been differentiated so far. Obviously,

somaclonal variants and mutants cannot be separated from the original cultivar. The set of markers was expanded to twelve to give an added level of discrimination. All potato varieties maintained by SASA have been fingerprinted. SASA's potato SSR profile database is currently not public. This well established method will be applied for this AEGIS project.

The ECPGR European Potato Cultivar Database [EPCD] (www.europotato.org) currently lists information on about 5,700 (presumable) different clones (incl. more than 4,100 cultivars as well as 166,000 observations) provided by 64 contributors. Those variety names that were used more than once for different genotypes (e.g. Gloria), are listed with year of release. When the identity is unclear then the abbreviation of the data donor is included in the name label.

The aim of this project is to use microsatellite genotyping to assist in setting up the AEGIS collection for potato cultivars by means of fingerprinting old potato clones with questionable identity, to confirm or correct cultivar names.

Materials and Methods

Criteria for the initial selection of samples

The study was foreseen to fingerprint a maximum of 500 potato samples, meaning that only a reduced part of the in European collections available varieties can be screened. Criteria were developed for choosing the material that deserves a high priority for this DNA test. The main demand of AEGIS is that the germplasm that will be included in the European Collection has as a prerequisite that the designating country uses its sovereign rights over the germplasm material in their collections) on request by the collection holder. EURISCO was used to identify the clones that fulfil this condition by selecting *Solanum tuberosum* varieties with a "1" in the field MLSSTAT, indicating the status of the germplasm concerning the MLS as these accessions have comparable pre-conditions. Unfortunately, countries like France, Spain and Italy had put no potato germplasm (yet) into the MLS and were therefore automatically excluded from the initial accessions selection.

The next criterion was that only old varieties <=1960 (arbitrary) were included, because identity errors are in particular expected in elder varieties. This selection was expanded with a few >1960 varieties with the same name as an elder clone (e.g. Apollo), to have fingerprints available in case of doubt.

The third criterion was that samples/accessions from countries of origin were preferred over other accessions, because it is considered that countries of origin are the most reliable source (of information and material) with the lowest chance of exchanges /human errors etc. Furthermore, the country of origin will be the first responsible for maintaining these germplasm accessions. A somewhat uncomfortable situation turns up when the country of origin (e.g. Italy) does not put an old variety into the MLS, whereas a genebank in another country (e.g. Germany) puts the Italian clone into the MLS. On the other hand, some countries like the Netherlands do not maintain a public potato variety collection. In the Netherlands CGN did not accept the responsibility of maintaining *in vitro* collections, because they are much more costly

than maintaining accessions in the form of botanical seed. A small common collection is therefore being maintained by the Dutch breeders. Several Dutch heritage varieties have been offered to the IPK genebank in Germany and were included in its collection.

Finally, in some cases old varieties were not included, because a profile of the cultivar was already available at SASA from earlier work.

Material selection

The more than 23.000 potato accessions (incl. Andean wild & cultivated spp.) listed in EURISCO was reduced to almost 9.000 *Solanum tuberosum* varieties, from which 4161 had been put into the MLS. Applying the criteria described above, a first selection of 441 clones from seven different countries was made. This list is available from the AEGIS Potato project website. The seven involved collection holders were invited to participate in the AEGIS potato project and to comment on the draft list. Switzerland increased its number of samples to 25. Ireland reported that its collection was already SSR genotyped by SASA and later donated 30 fingerprints to the AEGIS project. For phytosanitairy reasons Germany could provide only a part of the requested accessions. The four accessions requested from Estonia were provided by the Ukraine, which is therefore listed as donor country. Encouraged by the Swiss partner, France (INRA) provided a list of 100 clones in November 2012 that were intended to be put into the MLS in the near future. From that French list 28 clones were selected for the genotyping study. SASA included 111 heritage varieties from the UK in the study that were not genotyped before and donated 24 other fingerprints from earlier work.

DNA Extraction

The majority of accessions analysed in this study were either from leaf material (dried and fresh) or micro-plant material. Some accessions were supplied by the partners in the form of previously extracted DNA. For the plant material the extraction method detailed in Reid *et al*. (2009 & 2011) was used.

Microsatellite markers and PCR conditions

In addition to the nine markers used in three multiplex reactions as documented in Reid *et al*. (2009 & 2011) three further markers (STMS 1016, 1024 & 2022) were used in this study in an additional multiplex set (Table 1). PCR reaction was carried out in 10 μL volumes using Type-It Microsatellite PCR Kit (Qiagen). Cycling and genotyping conditions were the same as those documented in Reid *et al*. (2009 & 2011). The final primer concentrations for multiplex 4 were 1pmol/μL for each primer. Alleles were scored as present or absent and the data stored and analysed using BioNumerics v7.0 (Applied Maths).

Allele codes

The alleles have been determined on a 3500xl capillary sequencer. Allele size may shift somewhat depending on the equipment used. In general, length differences between alleles are more reliable than overall allele size estimates (Deemer & Nelson,

2010). To name the alleles, letter codes (A-Z) have been preferred over the fragment length. A higher letter code not necessarily reflects a longer fragment. The base pair lengths of the alleles measured by a 3500xl sequencer are displayed in Table 7.

The twelve SSR markers are expected to be positioned in non-coding regions of the DNA and not to be subject to any selection pressure. The length of the alleles has therefore no relationship with any trait of the variety. The alleles are inherited to the progeny and could be used to check if the presumed parents of a variety would fit. The total composition of alleles for a set of markers is called the profile of a specific genotype. Profiles are considered to be different when they vary for at least two alleles. In this study the individual alleles are not of direct interest. They are of interest however when variety names have been used more than once, in order to compare with the profiles of all possible parents, which might lead to the correct identity. In this study the profiles were used to indicate that clones are duplicates or differ. In this way old potato clones having unreliable/unknown variety names were compared with each other and the database of known profiles at SASA. Somaclonal variants and mutants cannot be separated from the original cultivar by this reduced set of markers. N.B.: In evolutionary studies the delta mu genetic distance (Ddm) for microsatellites is being used (amongst others), assuming alleles mutating in length under a strict stepwise mutation process (Goldstein *et al*. 1995). For varieties (resulting from crossings) the length of the alleles has no phylogenetic value. Furthermore, determining genetic distances between varieties was not the purpose of this study.

Results

A total of 379 accessions from eight countries (Table 2) were genotyped with 12 SSR markers. A full list of the accessions submitted for genotyping is presented in Annex 1. Additionally, fingerprints from in total 54 heritage varieties were donated by Ireland and the UK, so in total 433 accessions were included in the study. Twelve varieties were represented by more than one accessions (Table 3) and an additional 11 by the same name but had different identifiers (normally different dates e.g. Flora, Flora 1939 and Flora 1955) (Table 4). Cluster analysis revealed 397 different taxa with 27 taxa containing 2 or more accessions (Table 5). When compared with the complete SASA database, which contains >6000 fingerprints on potato clones from different sources, some additional matches were found (see Annex 2 for complete list and Annex 3 for genotypes). It would appear that there are a number of varieties that have been given different names in different countries (Table 6). For example All Blue, Blaue Schweden (ECPD list additional synonyms of Gfohler Blaue and Sharons Blue), Blue Congo, Blue Salad, Congo (Swedish sample), McIntosh Black, Russian Blue, Salad Blue and Shetland Beauty all yield identical profiles. All results have been made available at the AEGIS Potato project website: http://documents.plant.wur.nl/cgn/pgr/AEGISpotato/. A poster on the results was presented at the 19th triennial EAPR meeting in Brussels (Hoekstra *et al*. 2014).

Discussion

- Country representatives will select/nominate accessions from their national collections to become part of the virtual European Collection. In the first place they will select varieties that originated from their own country. For some clones with unclear identity and/or origin (like Unbekannte Schwarze and Tennaer) the results from this study will be of great help, because the correct identities have now been determined. In other cases, clones with the same name appear to be definitely different (e.g. the two Iris and the two Shetland Wonder clones). In such cases all clones should be maintained and could be become part of AEGIS, but the identities require further investigation. The Potato Pedigree Database (Hutten & van Berloo 2001) lists for example three different varieties with the name Iris (from 1915, 1936 & 1977), so the name Iris could be correct for both clones. The year of first release and the corresponding pedigree should be different then. The profiles of the three Alma clones in this study are definitely different (meaning that they differ for at least two alleles) and can all three be part of the AEGIS collection, but are also rather similar and might be the progeny of the same crossing. The Potato Pedigree Database lists four different Alma's (from 1904, 1928, 1978 & 1984) with different pedigrees, so one would expect larger differences, unless the parents are all quite similar too.
- At the start of the project it was assumed that where names have been used more than once, the profiles of the parents might lead to the correct identity. For the duplicate group Fortuna/Morgane_1985 in this study it is unclear which one has been mislabelled. The Potato Pedigree Database lists four different varieties for Fortuna (from 1893, <1950, 1950 and 1981) with different pedigrees. Naktuinbouw (Netherlands Inspection Service for Horticulture) has profiles of 4 out of 10 Fortuna and Morgane_1985 parents, which were received from SASA. Compared with the Fortuna/Morgane_1985 profile, Marijke, Eigenheimer, Manna and Arran Pilot all fit as a parent, so the identity remains unclear. BF 15 does not fit as a parent, meaning that the profile is definitely not from Morgane_1955, but this was not the question. This small exercise shows that profiles of parents may not or only partly be available and that for genotypes containing only the more common alleles several varieties would fit as a parent. This leads to the conclusion that for heritage varieties, containing mostly only the more common alleles, under the circumstances of using a rel. limited set of SSR markers (in this study 12), the available profiles of possible parents may not be able to resolve the identity. Still it is worth checking, as some of the presumed identities may be excluded.
- For clones with unclear origin country or from countries that do not have a variety collection or maintain only a non-public or small collection (Netherlands) or that did not put their accessions into the MLS (Italy, Spain, France) it remains unclear which country/collection will put such germplasm into the AEGIS collection. The ECPGR Secretariat (J. Engels) suggests to first include 'reserve accessions' and to replace these at a later stage when the country of origin includes those accessions in the European Collection. For example at 3 March 2014 Italy has joined AEGIS and it will most probably include the Italian potato varieties.
- In order to identify internal redundancy, several collection holders are fingerprinting their potato clones with SSRs (Droz *et al*. 2012, Diekmann & Dehmer 2014, Esnault *et al*. 2014, Marhadour *et al*. 2014). Mostly with a deviant set of markers, which may be caused by the suitability of the marker/equipment combination. Internal redundancy will be identified in the different studies and the

use of (partially) different marker sets will most probably lead to comparable results when the same set of clones would have been screened. However, a direct comparison of the profiles between the different studies is only possible for the limited number of identical markers, meaning that an overlap of e.g. only three markers might not be informative enough and indicate many (false) redundancies. So far a comparison with profiles produced in other countries is lacking, apart from the cooperation between France and Switzerland and the recent cooperation between the UK (SASA) and the Netherlands (Naktuinbouw). Such an international comparison would provide important additional data for AEGIS. The current study emphasizes the need. The results from the current study can directly be used by the curators, but for the other accessions the overlap in the used set of markers between labs should be increased, preferably to at least a standard set of markers.

- In order to be able to harmonize the available results in different labs a standard set of genotypes could be scored. For harmonization the set does not require to contain all alleles. For those who are interested a minimum set of 35 genotypes, out of 7085 clones, was selected by A. Reid. This set contains all 126 alleles known at SASA, including the rare alleles. The set has only three genotypes in common with the AEGIS study, because heritage varieties hardly contain rare alleles.

Recommendations

1. It would appear that a number of varieties have been given different names in different countries (e.g. Eigenheimer in the Netherlands and Tennaer in Switzerland). It would be of great benefit for the European Collection if these were recorded as synonyms.
2. During the course of the analysis it became also apparent that there are some varieties that have been miss-labelled. Collection holders should resolve these issues. In case the correct identity of a clone having a unique profile is unknown (e.g. for the Congo clone in the UK) then the variety should be grown and the plant morphological described according to DUS standards. This description including the sprout colour assessment results might lead to its correct identification.
3. Several collections are currently fingerprinting their potato accessions with SSRs. Efforts should be made to allow comparison of the fingerprints, where possible. The latest news from CPVO (Reid pers. comm. 2014) is, that all new varieties going through DUS in Europe will be fingerprinted as part of the DUS test as governed by CPVO, using the 9 markers from the CPVO study (Reid *et al*. 2009 & 2011). These 9 markers have also been used in this AEGIS study (Table 1, multiplex sets 1–3). This recent decision of CPVO will presumably lead to the establishment of a central SSR-fingerprint database. Research labs should include at least these 9 markers, to have compatible results with the future CPVO SSR-fingerprint database and enable a comparison of the their results with those from other potato collections. It is unclear if the CPVO SSR-fingerprints will become public, but the national database managers (e.g. from Naktuinbouw or SASA) will be willing to seek matches to a specific profile. For elder varieties there may be no profile available in the database.

Bibliography

Deemer D.L. and C.D. Nelson (2010) Standardized SSR allele naming and binning among projects. BioTechniques 49: 835-836.

Diekmann, K. and K.J. Dehmer (2014) Molecular investigation of IPK's potato collections at a large scale. In: 19th triennial conference EAPR 2014, Brussels.

Droz, E., S. Ulliel, C. Julmi-Moreillon, S. Dorsat, J.-P. de Joffrey, D. Thomas, C.-L. Lê and K. Gindro (2012) Charakterisierung von Pflanzensorten via genetisches Profil. Agrar Forschung Schweiz, Heft 11-12: 552-553.

Esnault F, J. Solano, R. Pellé, J.P. Dantec, C. Souchet, M.C. Kerlan, P. Brabant, J.E. Chauvin (2014) Molecular analyses of the potato collection maintained in the INRA BrACySol Biological Resource Center. In: 19th triennial conference EAPR 2014, Brussels.

European Cultivated Potato Database (www.europotato.org).

Frese, L. & R. Hoekstra (2009) Report on a Network Coordinating Group on Sugar, Starch and Fibre Crops. Third Meeting, 8-9 October 2009, Quedlinburg, Germany www.ecpgr.cgiar.org/fileadmin/bioversity/publications/pdfs/1531_Sugar_Starch_Fibre_Crops_NCG_3rd_meeting_Quedlinburg_final.pdf

Ghislain, M., Núñez, J., del Rosario Herrera, M., Pignataro, J., Guzman, F., Bonierbale, M. and Spooner, D.M. (2009) Robust and highly informative microsatellite-based genetic identity kit for potato. Molecular Breeding 23: 377-388.

Goldstein, D.B., A. Ruíz-Linares, L.L. Cavalli-Sforza and M.W. Feldman (1995) An evaluation of genetic distances for use with microsatellite loci. Genetics 139: 463-471.

Hoekstra, R., A. Reid, K.J. Dehmer, J. Domkárová, S. Solberg, G. Doherty, J.-E. Chauvin, E. Droz, K. Kotkas, I. Skrabule (2014) SSR genotyping of old potato varieties to identify mislabelling & synonyms for AEGIS. In: Abstracts Book, Proceedings of Conference of the 19th Triennial Conference of the EAPR. Brussels, Belgium, 6-11 July 2014. p. 150.

Hutten R.C.B. and R. van Berloo (2001) An online Potato Pedigree Database. URL: www.plantbreeding.wur.nl/potatopedigree/

Kawchuk, L.M., Lynch, D.R.,Thomas, J., Penner, B., Sillito, D. and Kulcsar, F. (1996) characterization of Solanum tuberosum simple sequence repeats and application to potato cultivar identification. American Potato Journal 73: 325-335.

Marhadour S, A. Mear, C. Dargier, N. Laversin, M. Perramant, V. Pavy, F. Meytraud, G. Bronsard, F. Esnault, Y. Le Hingrat (2014) Seed potato identification using SSR markers in France: orginization, methods and database. In: 19th triennial conference EAPR 2014, Brussels.

Milbourne, D., Meyer, R.C., Collins, A.J., Ramsey, L.D., Gebhardt, C. and Waugh, R. (1998) Isolation, characterisation and mapping of simple sequence repeat loci in potato. Molecular and General Genetics 259: 233-245.

Reid, A., Hof, L., Esselink, D. and Vosman, B. (2009) Potato cultivar genome analysis. In Methods in molecular biology, plant pathology, vol.508, ed. R. Burns, 295–308. New York: Springer.

Reid, A., Hof, L., Felix, G., Rücker, B., Tams, S., Milczynska, E., Esselink, D., Uenk, G., Vosman, B. and Weitz, A. (2011) Construction of an integrated microsatellite and key morphological characteristic database of potato varieties on the EU Common Catalogue. Euphytica 182: 239-249.

Attachments

Annex 1: Origin of samples
Annex 2: Heritage variety observations
Annex 3: Heritage variety alleles

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Table 1. Marker information showing the repeat motif of the microsatellite, linkage group (or chromosome) and original reference.

Multiplex set	**Marker name**	**Repeat motif**	**LG**	**Reference**
1	STMS 0019	$(AT)_7$ $(GT)_{10}$ $(AT)_4$ $(GT)_5$ $(GC)_4$ $(GT)_4$	VI	Milbourne *et al*., 1998
	STMS 3009	$(TC)_{13}$	VII	Milbourne *et al*., 1998
	SSR1	$(TCAC)_n$	VIII	Kawchuk *et al*., 1996
2	STMS 2005	$(CTGTTG)_3$	XI	Milbourne *et al*., 1998
	STMS 3012	$(CT)_4.(CT)_8$	IX	Milbourne *et al*., 1998
	STMS 3023	$(GA)_9.(GA)_8.(GA)_4$	IV	Milbourne *et al*., 1998
3	STMS 2028	$(TAC)_5.(TA)_3.(CAT)_3$	XII	Milbourne *et al*., 1998
	STMS 5136	$(AGA)_5$	I	Ghislain *et al*., 2004
	STMS 5148	$(GAA)_{17}$	V	Ghislain *et al*., 2004
4	STMS 1016	$(TCT)_9$	VIII	Milbourne *et al*., 1998
	STMS 1024	$(TTG)_6$	VIII	Milbourne *et al*., 1998
	STMS 2022	$(CAA)_3..(CAA)_3$	II	Milbourne *et al*., 1998

Table 2. Number of accessions submitted by each country analysed in this study.

Country of submission	**Number of accessions**
Czech Republic	39
France	28
Germany	136
Latvia	2
Sweden	34
Switzerland	25
Estonia (material provided by Ukraine)	4
Ireland	0 (+ 30 donated fingerprints)
United Kingdom	111 (+ 24 donated fingerprints)

Table 3. Varieties with more than one isolate. N.B. Two samples of Atlas with the same accession number were submitted and both were processed to ensure conformity.

Variety	**Accession #**	**Country of submission**
Alma	07S0100022	Czech Republic
Alma (?)	12682	Germany
Alma	P073	Switzerland
Arran Cairn	11264	Germany
Arran Cairn	AEG-0215	United Kingdom
Atlas (1960)	10775-1	Germany
Atlas (1960)	10775-2	Germany
Aura	SOL000427	France
Aura	06/308	United Kingdom
Bishop	12256	Germany
Bishop	AEG-0211	United Kingdom
Early Rose	P035	Switzerland
Early Rose	07/530	United Kingdom
Flaminia	SOL000581	France
Flaminia	10381	Germany
Iris	11515	Germany
Iris	AEG-0183	United Kingdom
Monika	07S0102097	Czech Republic
Monika	07S0101517	Czech Republic
Robijn	10606	Germany
Robijn	AEG-0240	United Kingdom
Sefton Wonder	10030	Germany
Sefton Wonder	AEG-0200	United Kingdom
Vitelotte	12317	Germany
Vitelotte	P026	Switzerland

Table 4. Varieties with more than one isolate with different identifiers.

Variety	**Accession number**	**Country of origin/ submission**
Anna	07S0100043	Czech Republic
Anna (1947)	10784	Germany
Astra	07S0100078	Czech Republic
Astra	AEG-0044	Latvia
Astra (1983)	10225	Germany
Athene	07S0100080	Czech Republic
Athene (1964)	10543	Germany
Flora	07S0102066	Czech Republic
Flora	07S0100355	Czech Republic
Flora (1939)	10837	Germany
Flora (1955)	10845	Germany
Gabi	07S0100383	Czech Republic
Gabi (1989)	11462	Germany
Luna (1954)	10372	Germany
Luna (1998)	12669	Germany
Morgane-1955	SOL000727	France
Morgane-1985	SOL000726	France
Orion	SOL000278	France
Orion	AEG-0192	United Kingdom
Orion (Schots)	12436	Germany
Palma (1951)	10829	Germany
Palma (1972)	11717	Germany
Petra (1958)	10375	Germany
Petra (1991)	12349	Germany
Regent	07S0100850	Czech Republic
Regent (NLD)	10437	Germany

Table 5. Matching accessions from those submitted.

Variety	**Accession number**	**Country of submission**
Centrifolia	08/062	United Kingdom
Rosafolia	P067	Switzerland
Atlas (1960)	10775-1	Germany
Atlas (1960)	10775-2	Germany
Adelheid	12139	Germany
Oberambacher Adelheid	10522	Germany
Aura	SOL000427	France
Aura	06/308	United Kingdom
Gabi	07S0100383	Czech Republic
Gabi (1989)	11462	Germany
Lauterbrunnen	P028	Switzerland
Robijn	10606	Germany
Robijn	AEG-0240	United Kingdom

Flora	07S0102066	Czech Republic
Flora	07S0100355	Czech Republic
Flora (1955)	10845	Germany
Flaminia	SOL000581	France
Flaminia	10381	Germany
Monika	07S0102097	Czech Republic
Monika	07S0101517	Czech Republic
Ulster Premier	08/029	United Kingdom
Red Ulster Premier	08/028	United Kingdom
Ryecroft Purple	AEG-0238	United Kingdom
Shetland	AEG-0172	United Kingdom
Bleue d'Auvergne	SOL000458	France
Blaue Österreich	AEG-P053	Switzerland
Karjalan Musta	3375	Sweden
Skerry Blue	12037	Germany
Anna	07S0100043	Czech Republic
Anna (1947)	10784	Germany
Early Rose	07/530	United Kingdom
Puritan	07/570	United Kingdom
Peachbloom	AEG-0243	United Kingdom
Rödbrokig svensk	3062	Sweden
Bishop	12256	Germany
Bishop	AEG-0211	United Kingdom
Orion	AEG-0192	United Kingdom
Orion (Schots)	12436	Germany
Congo	3312	Sweden
Blaue Schweden	P017	Switzerland
Regent	07S0100850	Czech Republic
Regent (NLD)	10437	Germany
Eigenheimer	AEG-0170	United Kingdom
Blauwe Eigenheimer	12145	Germany
Tennaer	P024	Switzerland
Arran Cairn	11264	Germany
Arran Cairn	AEG-0215	United Kingdom
Astra	AEG-0044	Latvia
Astra (1983)	10225	Germany
Blaue Veltlin	P025	Switzerland
Blue Peter	AEG-0209	United Kingdom
Unbekannte Schwarze	07S0101998	Czech Republic
Vitelotte	P026	Switzerland
Vitelotte	12317	Germany
Viteotte Noire	SOL000366	France

Table 6. Varieties matching 3 or more other varieties. [1] Varieties submitted for testing in this study. [2] The two samples of Congo yield different profiles (ECPD lists two varieties named Congo one form the UK and the other from Sweden).

Varieties with same fingerprint	**Country that provided accession**
Acht-Wochen-Nüdeli[1]	Germany
Asparges	Netherlands & United Kingdom
Banana	Canada
Naglerner Kipfler	Germany & Netherlands
Ratte	Netherlands & United Kingdom
Argyll Blue	United Kingdom
Arran Victory	United Kingdom, Ireland & Canada
Blaue Österreich[1]	Switzerland
Bleue d'Auvergne[1]	France
Karjalan Musta[1]	Sweden
Orkney Blue	United Kingdom
Skerry Blue	Germany
All Blue	Canada
Blaue Schweden[1]	Switzerland
Blue Congo	United Kingdom & Canada
Blue Salad	United Kingdom
Congo[1&2]	Sweden
McIntosh Black	Canada
Russian Blue	United Kingdom & Canada
Salad Blue	United Kingdom
Shetland Beauty	United Kingdom
Blauwe Eigenheimer[1]	Germany
Eigenheimer	Ireland & United Kingdom
Northern B	United Kingdom
Tennaer[1]	Switzerland
Blaue Veltlin[1]	Switzerland
Blue Peter	United Kingdom
Congo[2]	United Kingdom & Ireland
Unbekannte Schwarze[1]	Czech Republic
Vitelotte[1]	United Kingdom, Germany & France
Viteotte Noire[1]	France
American Rose	United Kingdom
Beauty of Hebron	Ireland & Canada
Early Rose	United Kingdom & Canada
Early Puritan	United Kingdom
Puritan	United Kingdom
Duke of York	Ireland & United Kingdom
Eersteling	Netherlands
Fjellfinn[1]	Sweden
Pink Duke of York	United Kingdom
Rode Eersteling	Netherlands & United Kingdom

Table 7. Fragment length of 126 alleles from twelve SSRs on a 3500xl capillary sequencer.

Allele	0019	1016	1024	2005	2022	2028	3009	3012	3023	5136	5148	SSR1
A	167.1	234.4	138.3	153.1	166.1	286.5	142.1	165.3	176.8	213.7	402.8	201.5
B	193.2	241.4	141.8	159.5	178.1	295.4	146.6	167.3	178.8	216.4	416.6	204.7
C	197.0	243.4	145.1	165.6	181.1	365.4	150.5	195.4	186.6	219.4	422.7	206.7
D	199.3	246.5	145.9	171.7	184.2	388.2	153.1	197.4	196.1	225.3	425.2	208.8
E	204.4	247.6	148.9	184.2	192.7	395.1	155.1	199.8		228.0	428.1	212.7
F	208.4	249.4	152.4	196.3	221.4	401.0	159.4	201.2		231.0	431.9	214.8
G	235.5	250.5	155.5		233.1	405.5	165.5	211.6		236.5	433.3	216.8
H	175.3	255.6	158.1			408.1	167.5			248.2	440.2	219.0
I	169.2	256.6				292.8	171.0			250.7	446.4	220.7
J	181.5	258.5					175.7			245.0	450.7	224.8
K		259.6					157.6			242.0	452.8	228.7
L		261.6					161.4			222.0	457.2	202.9
M		262.7					173.8				461.6	223.0
N		265.8					152.1				464.6	210.7
O		268.8									471.0	
P		253.7									475.1	
Q											477.4	
R											420.5	
S											442.6	
T											473.4	