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INTERNATIONAL UNION FOR THE PROTECTION OF NEW VARIETIES OF PLANTS

GENEVA

AD HOC CROP SUBGROUP ON MOLECULAR TECHNIQUES FOR POTATO

Second Session
Quimper, France, April 17, 2007

ADDENDUM TO DOCUMENT BMT-TWA/POTATO/2/2

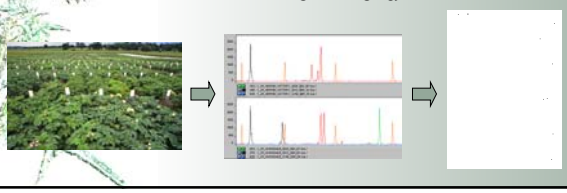
IDENTIFICATION OF POTATO CULTIVARS ON THE EUROPEAN UNION COMMON CATALOGUE USING SIMPLE SEQUENCE REPEAT (SSR) MARKERS

Document prepared by an expert from the United Kingdom

This document is an addendum to document BMT-TWA/Potato/2/2 "Identification of Potato Cultivars on the European Union Common Catalogue using Simple Sequence Repeat (SSR) Markers" and contains a copy of the presentation made by Mr. Alex Reid, United Kingdom, at the second session of the *Ad Hoc* Crop Subgroup on Molecular Techniques for Potato.

Genotyping the European Union Common Catalogue for potato

Alex Reid



The background

24th edition of the EU Common Catalogue contains 1086 varieties from 27 countries.

In order to pass Distinctness part of the DUS test a new variety must be compared to all varieties of 'common knowledge'.

For many testing stations the maintenance of living reference collections is rapidly becoming impractical due to the large numbers of varieties in terms of space and cost.

Many characters are quantitatively expressed and can be influenced by environmental factors.

Morphological descriptions can also be subjective and can vary between testing stations.

This situation is only going to get worse!

The project

To produce a database containing data for SSRs and key morphological features for the potato varieties on the EU Common Catalogue.

Co-funded by The Community Plant Variety Office.

Scottish Agricultural Science Agency (SASA), United Kingdom

Naktuinbouw, The Netherlands

Bundessortenamt (BSA), Germany

Centralny Ośrodek Badania Odmian Roślin Uprawnych (COBORU), Poland

System harmonization

Two of the partners have the capability to perform SSR analysis (UK & Netherlands).

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

However both use slightly different machines, polymers, etc. so allele sizes not exactly the same.

Reference varieties which contained all of the possible alleles known at that time were analysed at both sites and used to standardize the system. Alleles now assigned a letter and scored as binary data.

Morphological data stored in multi state format based on UPOV scoring system.

Allele scoring

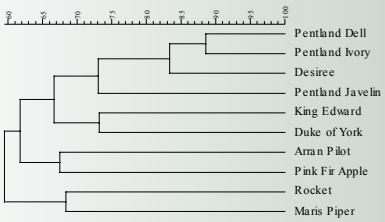
These data are stored in Access database showing presence (1) or absence (0) for each allele to give each variety an 89 digit 'telephone number'

Variety	A	B	A	B	C	D	A	B	C	D	A	B	C	D
Golden Wonder	1	1	0	1	1	0	1	1	0	1	1	1	1	1
Kerr's Pink	1	1	1	1	1	1	1	0	1	0	0	1	1	0

How many markers to use?

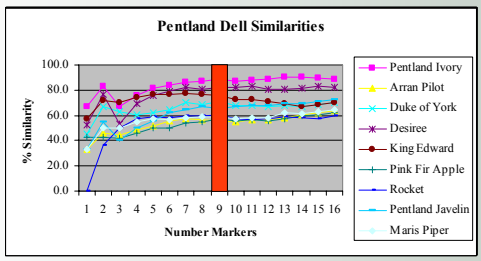
Marker	Number Alleles	Alleleic Phenotypes
2022	7	2
1024	8	4
3009	13	4
3023	4	4
LEMAX	4	4
1106	8	5
2005	6	5
2028	9	6
3012	7	6
5136	10	6
1104	9	7
1016	16	8
1105	12	9
5127	7	9
SSR1	13	9
5148	18	10

How many markers to use?



Tree obtained with 16 markers

How many markers to use?



The SSRs and morphological characters

SSRs
0019
2005
2028
3009
3012
3023
5136
5148
SSR1

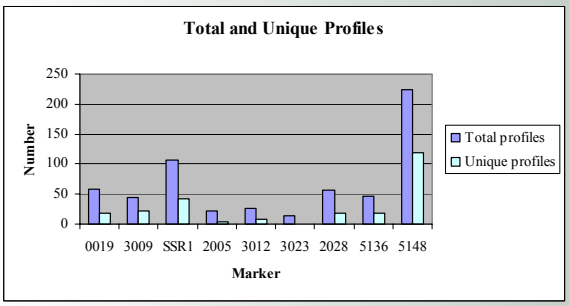
The SSRs and morphological characters

SSRs	Lightsprout
0019	Size
2005	Shape
2028	Intensity of anthocyanin colouration of base
3009	Pubescence of base
3012	Size of tip in relation to base
3023	Habit of tip
5136	anthocyanin colouration of tip
5148	Pubescence of tip
SSR1	Number of root tips
	Length of lateral shoots

The SSRs and morphological characters

SSRs	Lightsprout	Technical Questionairre
0019	Size	Plant: frequency of flowers
2005	Shape	Flower corolla: intensity of anthocyanin colouration on inner side
2028	Intensity of anthocyanin colouration of base	Flower corrolla: proportion of blue in anthocyanin colouration on inner side
3009	Pubescence of base	Plant: time of maturity
3012	Size of tip in relation to base	Tuber: shape
3023	Habit of tip	Tuber: colour of skin
5136	anthocyanin colouration of tip	Tuber: colour of base of eye
5148	Pubescence of tip	Tuber: colour of flesh
SSR1	Number of root tips	
	Length of lateral shoots	

Marker information (700 varieties)



The database

Integrated package that allows all types of morphological and molecular data to be analysed together. Can be linked via ODBC to Access. So far the database contains:

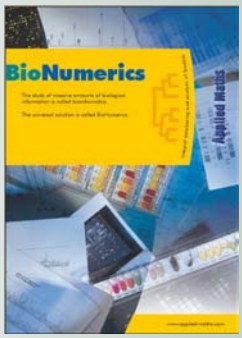
Over 1,000 varieties (821 from EUCC)

669 from single collections

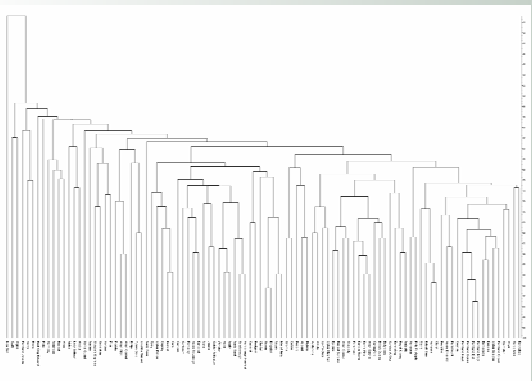
152 varieties from multiple collections

several sets of varieties and their mutants

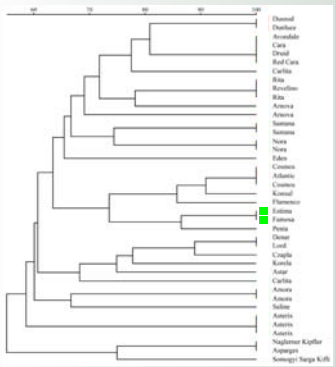
Library of reference varieties has been created to identify unknowns



National List - United Kingdom



Things you find out about your collections!



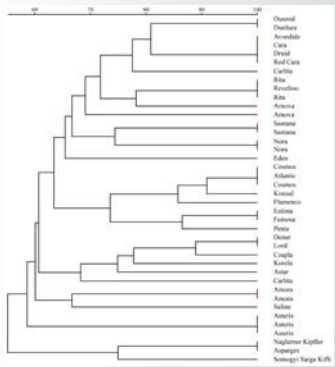
The Good

The majority of varieties appear to be correct

This includes sets of varieties and somaclonal mutants

Around 93% of all varieties from multiple sources match each other 100%

Things you find out about your collections!



The Bad

Some pairs yield sufficiently different profiles that it is obvious that one of them is incorrectly labelled but we don't know which one!

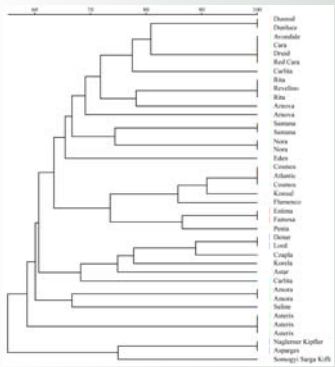
Other samples and/or morphological studies will hopefully reveal which one is the true variety and may also identify the 'rogues'

Around 5% of all pairs of varieties fall into this category

Allelic differences between mismatched varieties

Variety (source)	0019	3009	SSR1	2005	3012	3023	2028	5136	5148	% similarity
Carlita (NL)	DFG	FG	AF	ABD	BC	AD	ACD	CEF	BJOP	63.8
Carlita (UK)	BF	BG	ADI	ADF	BC	ABD	AB	EFH	JOP	
Arnova (NL)	F	G	DI	BD	BCF	AD	ABC	EF	IJ	82.1
Arnova (UK)	F	FG	DI	ABD	BF	AB	ABC	CEF	IJO	
Fresco (NL)	BG	BDG	ADI	D	BC	AB	A	EF	CIJ	100.0
Fresco (PL)	BG	BDG	ADI	D	BC	AB	A	EF	CIJ	
Fresco (UK)	BF	FG	DI	ABDF	B	BD	AC	DF	IJOP	55.0

Things you find out about your collections!



The Ugly

Some have been incorrectly labelled (Revelino, Atlantic, Dunrod and/or Dunluce)

One pair (Denar & Lord) have the same parents but different morphologies. It is unclear if this result is a mix up or shortfall of the system. These will be re-tested this year

Some have inconclusive morphological data (e.g. Naglerner Kipfler and Asparges)

Problems

Some varieties match things that they shouldn't e.g.
Corine (UK) matches Concurrent (UK and NL)
Spunta (NL) matches Slaney (UK and NL)
Revelino (UK) matches Rita (UK and NL)
Atlantic (UK) matches Cosmos (UK and NL)
Latona (PL and NL) matches Mirta (UK)

Some varieties differ between collections and don't match anything else e.g.
Allerfrüheste Gelbe, Amanda, Karatop, Cleopatra, Junior, Kuras

Some match but (maybe) shouldn't e.g.
Denar and Lord
Naglerner Kipfler and Asparges

However,

The vast majority of varieties are clearly distinguishable on the basis of these 9 SSR markers and problems with identification are, so far, entirely due to mislabelling.

Applications of the system

Identification of potato varieties for:
- culture collections
- breeders
- farmers
- regulatory bodies

Extensively used during trace-back investigations during recent ring rot outbreak in the UK. (Has lead to revision of EU directive 98/85 which now recommends that infected varieties should be identified as part of the process in determining the extent of any future disease outbreak.)

Identification of potato varieties in samples of crisps, chips and dehydrated potato products as part of food authentication studies.

Summary

Identification system based on 9 SSR markers.

Rapid method - from plant to identification can be achieved in a single day as most of the process can be automated.

Any part of plant can be used for identification.

Testing of unknown samples results in accurate identifications.

To date over 1000 varieties entered in database.

Morphological data and lightsprout photographs still to be entered.

Remaining varieties are being sourced from their official maintainers for addition.