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

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Introduction

1. The 24th Edition of the European Union Common Catalogue (EU Common Catalogue) for potato contains approximately 1100 varieties from 27 countries. To be granted Plant Breeder's Rights (PBR) a new variety has to meet the criteria for Distinctness, Uniformity and Stability (DUS). The distinctness criterion requires that a variety must be clearly distinguishable from any other variety whose existence is a matter of common knowledge at the time of the filing of the application ("varieties of common knowledge"). Due to the increasing numbers of varieties of common knowledge, it is clearly becoming difficult for examination offices to maintain such large collections to cover the distinctness part of the test. There are also inherent problems with the maintenance of large collections as human error can easily occur and, if such mistakes were to be made, could be extremely difficult to detect.

2. Furthermore, DUS testing and variety descriptions are based on morphological and physiological characteristics. Many of the characteristics are quantitatively expressed and can be influenced by environmental factors. The combined effect of environmental influences and interpretation differences between observers from different examination offices is a fundamental limitation for the exchange of variety descriptions between examination offices and the setting up of a useful international database based on morphological descriptions.

3. At the beginning of 2006 the CPVO funded a project to construct an integrated database containing microsatellite and key morphological characteristics for the potato varieties in the EU Common Catalogue. The four partners involved in the project are responsible for DUS testing in Germany (DE), Netherlands (NL), Poland (PL) and the United Kingdom (GB). The database currently contains the data for nine SSR markers for several hundred varieties.

Materials and Methods

4. DNA was extracted from tuber, lightsprout or leaf material from varieties on the EU Common Catalogue using a slightly modified protocol with GeneScan lysis buffer (Abgene). The material was sourced from either the four partners' collections or, where possible, from the official maintainers themselves.

5. The SSRs from nine markers were amplified in three multiplex reactions (Table 1) and run on two different Applied Biosystems capillary electrophoresis platforms in the Netherlands and United Kingdom. Harmonization of allele scoring was achieved by running a limited set of varieties in the two laboratories and comparing the peaks obtained. Due to minor size differences caused by the use of the different platforms, alleles were assigned code letters for scoring purposes.

6. Alleles were scored in binary format as either present or absent in Excel and/or Access and then imported into a BioNumerics database (Applied Maths) for analysis.

Results

7. The nine SSR markers yielded a total of 89 alleles ranging from four for STM3023 to 18 for STM5148. The relative abundance of alleles was also reflected in the number of different and unique allelic phenotypes for each marker (Table 1).

Table 1. Information for the SSR markers used to differentiate potato varieties.

Multiplex set	*Marker*	*Linkage group*	*Number alleles*	*Allelic profiles*	*Unique profiles*
1	STM0019	VI	9	58	19
	STM3009	VII	13	45	22
	SSR1	VIII	13	107	42
2	STM2005	XI	6	23	5
	STM3012	IX	7	27	8
	STM3023	IV	4	14	1
3	STM2028	XII	9	56	19
	STM5136	I	10	47	19
	STM5148	V	18	223	118

8. Currently the database contains 781 of the varieties on the EU Common Catalogue, 159 of which have been sourced from more than one country (145 from two countries and 14 from three countries). There are also several sets of varieties and their somaclonal mutants, which yield identical results as would be expected. These multiple entries are particularly useful for assessing the validity of the database because, barring misidentification, the SSR results should match. Of the 159 multiple-sourced varieties, we encountered 32 varieties (20%) where the duplicates do not match (Table 2). The majority of these (22 varieties) can be explained by variety mislabeling, whilst the remaining 10 varieties do not match any other variety, and their identity will, hopefully, be resolved by morphology. For example, the varieties ‘Dunrod’ and ‘Dunluce’ (both from the United Kingdom collection) yield identical SSR profiles and, at present, it is not known which one is mislabeled. The Polish varieties ‘Denar’ and ‘Lord’ also yield identical results; however, both of these varieties resulted from a cross between the same parents lines (Z-79.36/20 x Z-79.554/810). No morphological data are available in the European Cultivated Potato Database (http://vapache/ecpd/menu.php). It is therefore unclear whether one is a somaclonal variant of the other, or if they are morphologically identical but have a physiological difference. The varieties ‘Asparges’ and ‘Naglerner Kipfler’ also yield identical SSR profiles; however, in this case, morphological descriptions are available for both varieties and prove to be very similar (Table 3), indicating that they may indeed be the same variety.

Table 2. Differences in alleles between multiple samples.

Variety (source)	*0019*	*3009*	*SSR1*	*2005*	*3012*	*3023*	*2028*	*5136*	*5148*	*% similarity*
'Sava' (NL)	BDFG	BG	FI	ABD	BD	ABD	AC	EF	AJO	60.9
'Sava' (GB)	BG	FG	ACD	AB	BF	ABD	BC	DEFH	IJO	
'Junior' (NL)	BDFG	DG	ADK	BD	BD	AB	AC	DEF	JOP	73.2
'Junior' (GB)	DFG	G	DI	BDF	B	ABD	A	DEF	IJOP	
'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' (GB)	BF	FG	DI	ABDF	B	BD	AC	DF	IJOP	55.0

Table 3. Morphological characteristic comparison of Asparges and Naglerner Kipfler

(average values of data from The European Cultivated Potato Database).

Characteristic	*'Asparges'*	*'Naglerner Kipfler'*
Maturity	6	6
Foliage cover	6	7
Flower colour	2	2
Flower frequency	5.3	3
Berries	3	1
Tuber skin colour	1	1
Tuber eye colour	1	1
Flesh colour	4	4
Tuber shape	5.8	6
Eye depth	5.8	7.5
Tuber size	4.25	6
Uniformity	5.7	6

Conclusions

9. A database has been constructed containing data from nine SSR markers for 781 varieties on the EU Common Catalogue. With a few exceptions (somaclonal variants and mislabeled varieties), all varieties can be differentiated using the nine markers.

10. The use of three multiplex, instead of nine simplex, PCR reactions has allowed high throughput and cost savings.

11. The inclusion of multiple samples from various sources for a number of varieties has demonstrated that errors do occur in the labeling of varieties held in large collections. This demonstrates the importance of this kind of technology as an aid to DUS testing.

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