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

*Paper prepared by experts from the United Kingdom*

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

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## Introduction

The 24th Edition of the European Union Common Catalogue for potato contains 1093 varieties from 25 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.

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.

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 European Union Common Catalogue (EU Common Catalogue). The four partners involved in the project are responsible for DUS testing in Germany, the Netherlands, Poland and the United Kingdom. The database currently contains the data for nine SSR markers for several hundred varieties.

## Materials and Methods

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.

The SSRs from nine markers were amplified in three multiplex reactions (Table 1) and run on two different Applied Biosystems 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.

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

Results

The nine SSR markers yielded a total of 88 alleles ranging from 4 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).

Currently, the database contains 579 of the 1093 varieties on the EU Common Catalogue. Including varieties that have come from more than one source the total number of entries is 675 (75 varieties from 2 sources and 11 varieties from 3 sources). There are also 4 sets of varieties and their somaclonal mutants. These multiple entries are particularly useful for assessing the validity of the database as, barring misidentification, the SSR results should match. Of the 75 pairs of varieties, 65 matched exactly (87%), 9 of the 11 triplicate samples matched (82%) and of the remaining 2 (Fresco and Latona), 2 of the 3 samples yielded an exact match. All 4 sets of varieties and their somaclonal mutants gave identical results as expected. In every case where varieties do not match when expected to do so there have been significant differences in their SSR profiles (Table 2) indicating that the difference is caused by sample mislabeling at some time in one (or more) of the collections. In the case of Fresco and Latona, the samples from the Netherlands and Poland match exactly whereas the United Kingdom samples are different suggesting that this may be the one that is incorrectly labeled. In a small number of cases, exact matches were observed between pairs of varieties where none might have been expected. For example, the varieties Dunrod and Dunluce (both from the United Kingdom collection) are identical 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) (The European Cultivated Potato Database http://vapache/ecpd/menu.php). Unfortunately, no morphological data is currently available for either of these two varieties so it is 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 and prove to be very similar (Table 3).

Conclusions

A database has been constructed containing data from 9 SSR markers for 579 of varieties on the EU Common Catalogue. With a few exceptions (somaclonal variants and mislabeled varieties) all varieties can be differentiated using the nine markers.

The use of 3 multiplex instead of 9 simplex PCR reactions has allowed high throughput and cost savings.

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.

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 | 57 | 20 |
| | STM3009 | VII | 13 | 36 | 16 |
| | SSR1 | VIII | 13 | 98 | 36 |
| 2 | STM2005 | XI | 6 | 20 | 6 |
| | STM3012 | IX | 7 | 26 | 8 |
| | STM3023 | IV | 4 | 14 | 3 |
| 3 | STM2028 | XII | 8 | 50 | 19 |
| | STM5136 | I | 10 | 41 | 13 |
| | STM5148 | V | 18 | 199 | 117 |

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 (UK) | BG | FG | ACD | AB | BF | ABD | BC | DEFH | IJO | |
| Junior (NL) | BDFG | DG | ADK | BD | BD | AB | AC | DEF | JOP | 73.2 |
| Junior (UK) | 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 (UK) | 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 |

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