



BMT/14/18 Add.

ORIGINAL: English

DATE: November 27, 2014

INTERNATIONAL UNION FOR THE PROTECTION OF NEW VARIETIES OF PLANTS

Geneva

WORKING GROUP ON BIOCHEMICAL AND MOLECULAR TECHNIQUES AND DNA-PROFILING IN PARTICULAR

Fourteenth Session
Seoul, Republic of Korea, November 10 to 13, 2014

ADDENDUM TO DOCUMENT BMT/14/18

A EUROPEAN POTATO DATABASE AS CENTRALIZED COLLECTION OF
VARIETIES OF COMMON KNOWLEDGE
(A CPVO FOLLOW UP PROJECT OF THE R&D PROJECT "CONSTRUCTION OF AN INTEGRATED
MICROSATELLITE AND KEY MORPHOLOGICAL CHARACTERISTIC DATABASE OF POTATO
VARIETIES IN THE EU COMMON CATALOGUE")

*Document prepared by experts from the Community Plant Variety Office of the European Union,
the United Kingdom and the Netherlands*

Disclaimer: this document does not represent UPOV policies or guidance

The Annex to this document contains a copy of a presentation "A European Potato Database as Centralized Collection of Varieties of Common Knowledge (A CPVO follow up Project of the R&D Project "Construction of an Integrated Microsatellite and key Morphological Characteristic Database of Potato Varieties in the EU Common Catalogue")" made at the fourteenth session of the Working Group on Biochemical and Molecular Techniques and DNA-Profiling in particular (BMT).

Alex Reid, Science and Advice for Scottish Agriculture (SASA), the United Kingdom (GB)

Hedwich Teunissen, Naktuinbouw, the Netherlands (NL)

Anne Weitz, European Union (EU), Community Plant Variety Office (CPVO)

Abbreviations used in the Annex;

| | |
|------------|--|
| EOs | Examination Offices |
| DB | Database |
| BSA, DE | Bundessortenamt, Germany |
| COBORU, PL | The Research Centre of Cultivar Testing, Poland |
| OEVV, ES | Spanish Plant Variety Office, Spain |
| DAF, IE | Department of Agriculture and Food, Ireland |
| AGES, AT | Austrian Agency for Health and Food Safety, Austria |
| UKZUZ, CZ | Central Institute for Supervising and Testing in Agriculture, Czech Republic |
| UKSUP, SK | The Central Controlling and Testing Institute in Agriculture, Slovakia |

[Annex follows]



A European Potato database as centralized collection of varieties of common knowledge

Alex Reid, SASA/GB
Hedwich Teunissen, Naktuinbouw/NL
Anne Weitz, European Union, CPVO

BMT/14, Seoul, 10-13.11.2014

Background info

A CPVO follow up project of the
R&D project

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



Limitations and risks of DUS system in potato

- Limited living reference collection:
- Limited coverage of databases (missing data):
- Limitations due to :
 - Distribution and maintenance (tubers) is expensive, risk for diseases.
 - quarantine regulations.
- Variation of morphology data:
 - Morphological observations and descriptions for same variety vary between EOs. Hard to exchange the descriptions.
 - Year-, location- and observer-effects.



The first DB

- DB contains only molecular data in BioNumerics
- Plus only limited lightsprout morphological data

Due to lack of harmonization between DUS-authorities
Study was limited to 4 examination offices in the EU

⇒ **Set up follow up project with 9 partners to complete the DB!**



Aim for an improved database

What:

- Improved quality of the procedure for potato DUS testing in EU

By:

- Harmonization (both morphology and markers)
- Improve the efficiency of the DUS testing
- Improve the management of the reference collection.



Aim

How:

- Construction of an integrated microsatellite and key morphological characteristic database of potato varieties in the EU Common Catalogue
- Combining (harmonized) morphological characteristics with (harmonized) genetic characteristics (microsatellites)



Aim

Who:

- **Harmonization exercises for morphological data and light sprout pictures for all responsible EOs in EU**
 - CPVO and 9 EU EOs: (Naktuinbouw (NL), SASA (GB), BSA (DE), COBORU (PL), OEVV (ES), DAF (IE), AGES (AT), UKZUZ (CZ), UKSUP (SK))
- **Harmonization of DNA data and synchronization of old profiles**
 - SASA (GB) and Naktuinbouw (NL)



Results on Morphological Harmonization

Ringtests with 8 varieties conducted at all 9 examination offices

- 2012 Meeting at Naktuinbouw/NL
- 2013 Meeting at SASA/GB
- 2014 Meeting at Bundessortenamt/DE
- ⇒ Identification of list of characteristics useful to enter DB
- ⇒ Harmonization of set up of lightsprouts cabinets
- ⇒ Define ownership, access rights and the use of DB results
- ⇒ Define contribution and maintenance of the DB

End of the project is foreseen end of 2015



Results on DNA Harmonization

All project partners submit plant material of their candidate varieties to SASA and Naktuinbouw to collect SSR profiles. For the duration of the project, the CPVO finances for the candidate varieties of each of the nine examination offices

- The transport to the lab
- Processing a sample and producing a fingerprint
- Analysis of profile with BioNumerics (looking for very similar varieties and matches)



Improved Common Potato DB

- Procedural aspects of the leaf sampling, DNA-extraction and DNA-analysis of potato candidate varieties for the growing season 2014 are described
- EO send their material to either GB or NL

| Year/EO | ES | IE | UK | NL | DE | AT | CZ | SK | PL | total |
|--------------|----|----|----|----|----|----|----|----|----|-------|
| 1. year | 2 | 5 | 13 | 46 | 27 | 5 | 7 | 3 | 23 | 131 |
| 2. year | 0 | 4 | 8 | 0 | 22 | 5 | 7 | 2 | 13 | 61 |
| 3. year | 0 | 0 | 0 | 0 | 2 | 2 | 1 | 0 | 0 | 5 |
| total | 2 | 9 | 21 | 46 | 51 | 12 | 15 | 5 | 36 | 197 |
| Send to lab: | UK | UK | UK | NL | UK | UK | NL | NL | NL | |



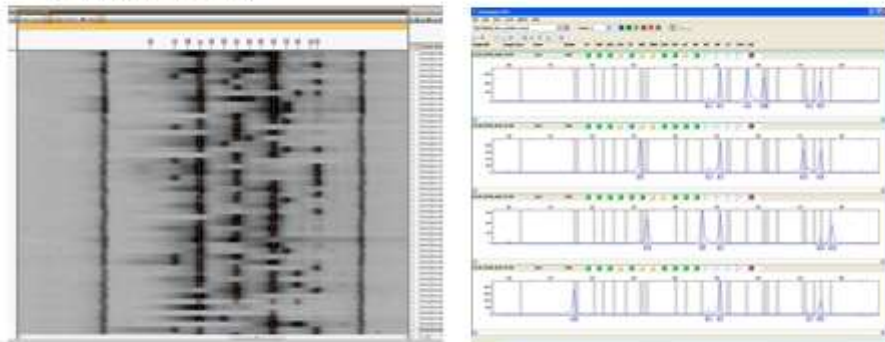
Improved Common Potato DB

- DNA was extracted and exchanged between the labs
- SSR profiles were generated for all samples in two labs
- The allele-scores were exchanged and checked for reproducibility
- Results (= 100% matches) will be reported to the CPVO and the responsible EO



Results of SSR analysis

- Reproducibility



- **Within** each lab reproducibility is high (100%)
- Reproducibility **between** labs is more challenging due to different platforms used



Results of SSR analysis

- **Reproducibility**

| Sample | SSR1 | SSR2 | SSR3 | SSR4 | SSR5 | SSR6 | SSR7 | SSR8 | SSR9 | SSR10 | SSR11 | SSR12 | SSR13 | SSR14 | SSR15 | SSR16 | SSR17 | SSR18 |
|--------|------|------|------|------|------|------|------|------|------|-------|-------|-------|-------|-------|-------|-------|-------|-------|
| 8100 | BD | BD | BD | BD | BD | BD | BD | BD | BD | BD | BD | BD | BD | BD | BD | BD | BD | BD |
| 8101 | CP | CP | BD | BD | BD | BD | BD | BD | BD | BD | BD | BD | BD | BD | BD | BD | BD | BD |
| 8102 | DU | DU | BD | BD | BD | BD | BD | BD | BD | BD | BD | BD | BD | BD | BD | BD | BD | BD |
| 8103 | DI | DI | BD | BD | BD | BD | BD | BD | BD | BD | BD | BD | BD | BD | BD | BD | BD | BD |
| 8104 | CP | CP | BD | BD | BD | BD | BD | BD | BD | BD | BD | BD | BD | BD | BD | BD | BD | BD |
| 8105 | DB | DB | BD | BD | BD | BD | BD | BD | BD | BD | BD | BD | BD | BD | BD | BD | BD | BD |
| 8106 | BD | BD | BD | BD | BD | BD | BD | BD | BD | BD | BD | BD | BD | BD | BD | BD | BD | BD |
| 8107 | CP | CP | BD | BD | BD | BD | BD | BD | BD | BD | BD | BD | BD | BD | BD | BD | BD | BD |
| 8108 | CP | CP | BD | BD | BD | BD | BD | BD | BD | BD | BD | BD | BD | BD | BD | BD | BD | BD |
| 8109 | DI | DI | BD | BD | BD | BD | BD | BD | BD | BD | BD | BD | BD | BD | BD | BD | BD | BD |
| 8110 | BD | BD | BD | BD | BD | BD | BD | BD | BD | BD | BD | BD | BD | BD | BD | BD | BD | BD |
| 8111 | BD | BD | BD | BD | BD | BD | BD | BD | BD | BD | BD | BD | BD | BD | BD | BD | BD | BD |
| 8112 | BD | BD | BD | BD | BD | BD | BD | BD | BD | BD | BD | BD | BD | BD | BD | BD | BD | BD |

Two types of problems:

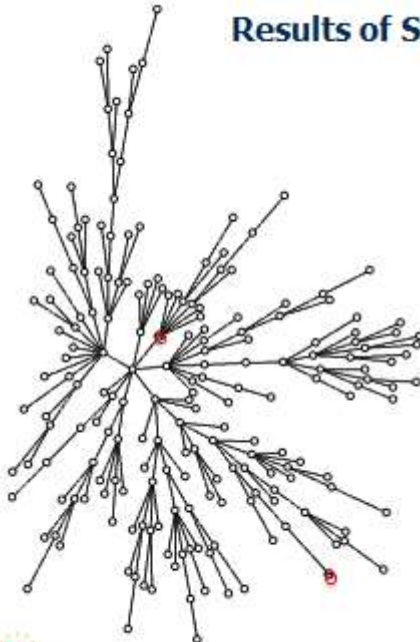
1. One lab calls a definite allele (present or absent) -
the other calls as questionable (is actually not a real problem)

2. Both labs different calls (a real problem)

Not *that* big an issue as differences are generally from a limited set of alleles that we already know can be a problem or alleles that are called as questionable by one of the labs. (We made scoring rules to overcome this minor problem)



Results of SSR analysis



- Tree for 2014 samples, unique apart from 2 pairs of samples
- one from NL the other from DE both (?) 1st year applications
- One from NL matched Agata from PL

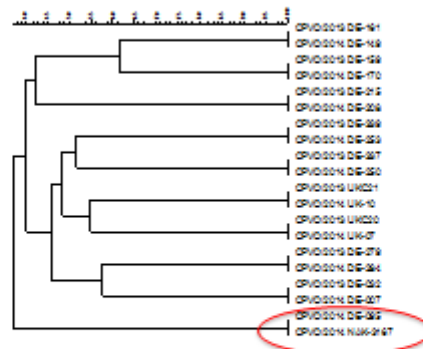


Results of SSR analysis

- If compare 2014 samples with the rest of the database
 - CPVO/2014 NAK-xxx matched with Agata.
 - CPVO/2014 ES-xxx matched with Zarina
 - CPVO/2014 DE-250 matched with DE sample from 2013 and with Abby (National Listing and Plant Breeders Rights for EU granted in 2013)
 - CPVO/2014 DE-237 matched with a NL candidate from 2013 of which the application was stopped.
-
- Occasionally (#3 in the last 3 years), we identify uniformity problems in candidates: testing two tubers revealed two different profiles



Results of SSR analysis



- 2013 and 2014 samples match as expected
- Matching pair from NL and DE



Problem analysis

- Samples tested in first year of application. Subsequent years samples only taken if there is any doubt
- What to do with matches of candidates from different EOs?
- What to do with candidates that match with a sample that was withdrawn in another country?
- Do we need to check for uniformity with DNA markers? No? If yes how many?



Future

- To keep the DB up to date we need information back from EOs concerning
 - Successful applications and subsequent **names**
 - Withdrawn samples
- Further investigation into how the combination of SSR and molecular characteristics could work
- Platform to share data with EOs and CPVO if desired (NL and GB already share SSR data)

