

Research & Development Project relevant to the Community Plant Variety System

## **Construction of a European Potato database with varieties of common knowledge and its implementation in the potato DUS testing system**

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### **Final Report**

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## **1. Introduction**

The development of an EU Potato DB started with a first CPVO co-financed R&D project in 2006-2008 carried out by UK, NL, DE and PL. Morphological descriptions, lightsprout pictures and molecular profiles with 9 SSR markers were collected for about 900 varieties from the EU Common Catalogue (CC). Due to limitations concerning harmonization of morphological descriptions and lightsprout pictures it was difficult to implement the DB in practical DUS testing. UK and NL continued on a national level to add SSR profiles of new candidate varieties applied in their countries to the DB in 2008-2011.

To enhance the setup of a common database to be used by all nine entrusted EOs, the CPVO initiated a ring test for harmonization of morphological descriptions. In parallel, SSR profiles of new candidate varieties applied with the four partners of the initial project were added to the DB.

CPVO and the four initial project partners agreed to open the DB to all nine entrusted examination offices. Subsequently SSR profiles of all candidate varieties tested in one of the EOs were added to the DB since 2014.

All EOs, the CPVO and ESA expressed the wish to continue the construction of an EU Potato DB and to implement such DB in order to improve the efficiency and quality of DUS testing in potato. It was agreed to use the GEMMA database software developed by GEVES, FR, in the framework of the CPVO co-funded R&D project "Management of peach tree reference collections". Further background information is summarized in section 1 of the project description (see Annex 1)

This new project was carried out with the following main objectives:

- Construction of the DB, definition of content
- Molecular profiling of new applications
- Collection and profiling of varieties of the CC which were still missing in the DB
- Development of procedures for feeding and maintaining the DB
- Implementation of the DB into the DUS systems of EOs
- Conclusion of agreements on continued contribution to the DB, rights and obligations of partners, confidentiality aspects

## **2. Development of GEMMA-Potato**

### **2.1 Construction of the common database**

Administrator access to the GEMMA software was given to EOs and CPVO at the beginning of the project. A half-day training on the use of the database was provided by GEVES in conjunction with the meeting in Hannover on 21.06.2016. The specific structure for the EU Potato DB was developed after the training. Appropriate import files have been agreed and implemented by all partners under their account (Standard GEMMA import files for administrative data, potato specific import files for guideline, characteristics, morphological data and molecular data). All partners used these files to upload own morphological data.

### **2.2 Information to be included**

It was agreed to include the following information in relation to varieties, administrative information, morphological descriptions, lightsprout pictures and molecular data:

### Varieties

	Morphology	Lightsprout pictures	DNA marker profile
Candidates (under test in that country)	X	X	X
Listed/protected in that country	X	(✓)	(✓)
CPVR based on test in that country	X	(✓)	(✓)
Listed/protected in other country (EO)	X	X	-
Listed/protected in other country (non-EO)	X	X	X

X – data to be generated and uploaded (✓) – already in DB (previous candidate / project)

- Candidates terminated before registration should be **deleted** from the DB (not in common knowledge)
- Listed/protected varieties should be **maintained** in the DB after termination (still in common knowledge even if no material available any more) – status TER or EX

### Administrative data

- Denomination
- Breeders Reference
- Country (defined via password access)
- Variety ID - identifier in the EO (alpha-/numerical)
- Present status (PRE, REG1, REG2, TER, EX)
  - PRE** variety under test; DNA profile available; no or 1<sup>st</sup> cycle morphological description
  - REG1** variety used in the reference collection because it is protected/listed in that country (incl. CPVR granted with report from that EO); morphological description is based on 2 or more cycles
  - REG2** variety not listed/protected in that country but considered in the reference collection as a variety of common knowledge (listed/protected in other countries); morphological description is based on 2 or more cycles
  - TER** variety not listed/protected in any country but still considered in the reference collection (material still available)
  - EX** variety is not used any more as a reference variety (no material available)
- Date of last modification (of the dataset in general; GEMMA)
- Unique Identifier (to indicate the same variety over EOs)

### Morphological data

All morphological data in the database have to be assessed after the ringtest (data for candidate and reference varieties from 2014 forward).

Agreed characteristics for inclusion in database:

CPVO TP 023/2	CPVO TP 023/3	Notes	Characteristic
4	4 G	1-3, VG, QN	Lightsprout: proportion of blue in anthocyanin colouration at base
8	8	1-9, VG, QN	Lightsprout: anthocyanin colouration of tip
12	12	1-3, VG, QN	Plant: foliage structure
13	13	1-9, VG, QN	Plant: growth habit
15	15	1-9, VG, QN	Leaf: outline size
16	16	1-5, VG, QN	Leaf: openness
17	17	1-9, VG, QN	Leaf: presence of secondary leaflets
20	20	1-9, VG, QN	Second pair of lateral leaflets: width in relation to length
23		1-9, VG, QN	Plant: height
28	27 G	1-9, VG, QN	Flower corolla: <u>intensity</u> of anthocyanin colouration on inner side
29	28 G	1-3, VG, QN	Flower corolla: proportion of blue in anthocyanin colouration on inner side
30	29	1-9, VG, QN	Flower corolla: <u>extent</u> of anthocyanin colouration on inner side
	30	1-5, VG, QN	Plant: height
32	32	1-6, VG, QN	Tuber: shape
33	33	1-9, VG, QN	Tuber: depth of eyes
34		1-7, VG, PQ	Tuber, colour of skin
	34 G	1-9, VG, PQ	Tuber: colour of skin
35	36	1-4, VG, PQ	Tuber: colour of base of eye
36	37	1-9, VG, PQ	Tuber: colour of flesh

### **Lightsprout pictures**

Guidance for lightsprout photos for inclusion into the EU Potato DB was developed and distributed to partners on 02/03/2017 (see Annex 2).

### **Molecular data**

Import files for inclusion of molecular data into the EU Potato DB were defined and distributed to partners on 26/09/2017.

The following SSR markers are used for the characterization of potato varieties in the project. Nine linkage groups are represented by one marker each. Three linkage groups are not covered by any marker.

Linkage group	Name	Repeat motif	Number of alleles <sup>*)</sup>	avg. diff. alleles per phenotype <sup>**)</sup>	PIC values <sup>**)</sup>
I	STMS 5136	(AGA)5	12	2.76	0.92
II	-				
III	-				
IV	STMS 3023	(GA)9.(GA)8.(GA)4	5	2.26	0.79
V	STMS 5148	(GAA)17	23	3.14	0.98
VI	STMS 0019	(AT)7 (GT)10 (AT)4 (GT)5 (GC)4 (GT)4	16	2.14	0.92
VII	STMS 3009	(TC)13	18	1.91	0.81
VIII	SSR1	(TCAC)n	15	2.81	0.91
IX	STMS 3012	(CT)4.(CT)8	8	2.25	0.87
X	-				
XI	STMS 2005	(CTGTTG)3	7	2.56	0.80
XII	STMS 2028	(TAC)5.(TA)3.(CAT)3	11	2.31	0.90

\*) Number of alleles as to February 2018

\*\*\*) Values calculated in CPVO R&D Potato project 2006-2008 (less alleles than 2018)

### 2.3 Experience with the database

In general, GEMMA was considered as a useful database with appropriate functionalities. It took some time to get practical experience with the GEMMA software and to understand the functionalities. The training was considered to be very useful. With the help of the manual, exchange between partners and individual support provided by GEVES all partners could upload the agreed data. Upload functionalities are easy and useful.

Some limitations have been identified in relation to similarity searches and output files. The following improvements were implemented into the software by GEVES on request of the coordinator until June 8<sup>th</sup>, 2018:

- The download of the similarity search results is possible for all datasets from other EOs now
- 'Present Status' and 'Type of description' were included in the download file.
- The notation of 'quantitative' and 'qualitative' data was adapted to common wording used in the UPOV community: 'measurement' and 'visual observation'.
- The download format of molecular data was modified ('0/1 matrix' instead of naming the present alleles).

Each EO can allow specific access to the own data per variety, EO and data type (phenotypic and/or molecular and/or picture). The structure of the access in GEMMA is very useful for individual settings. However, it was not obvious for the crop experts to understand from the manual which settings need to be made.

Most partners considered national procedures on downloaded data more appropriate for similarity searches than within GEMMA. In particular, GEMMA has limitations if similarity searches are done for a larger number of candidate varieties. National procedures are better adapted to the specific national needs, including links to other national DUS software.

The DB will be adapted by all partners to the new CPVO-TP/023/3. Descriptions according to TP/023/2 will be converted to CPVO-TP/023/3 for all unchanged characteristics.

### 3. Generation and management of molecular data

#### 3.1 Sampling of candidate varieties and missing varieties from Common Catalogue

A common procedure for sampling was agreed for 2016 and 2017. Sampling was continued in 2018 according to the same procedure (see Annex 3). All partners sent in two tubers of all new applications to one of the labs.

305 varieties from the common catalogue were identified to be missing in the database. They were allocated to the EOs in order to collect and sent material to the labs for analyses in 2017. Not all missing varieties could be obtained. Plant material was received and could be analysed for 209 varieties. Due to information received on the requests only very few of the varieties were again requested for 2018 (about 15 varieties).

#### Molecular profiling of missing CC varieties

	Analyzed	Missing	Remark on missing varieties
AT	21	5	no reply
CZ	38	8	not maintained
DE	10	10	1 not maintained; 9 no reply - new request for 2018
ES	32	23	20 no reply; 2 new request for 2018
IE	12	4	not maintained
NL	38	11	not maintained
PL	27	6	new request for 2018
SK	22	8	4 not maintained; 4 no reply
UK	9	21	no reply (RO)
	209	96	

IE – 10 varieties from SE received as micro-plants, tubers to be produced by EO

NL – due to phytosanitary restrictions only DNA received for 17 varieties from NO, no tubers

#### 3.2 Overview on generated molecular data

The following table provides an overview of the molecular profiling of varieties since the initiation of the first CPVO R&D project Potato I in 2006. NL and UK continued after the first project to analyse new candidate varieties applied in NL and UK. A follow up project was subsidized by the CPVO in order to continue profiling by be partners of the first project in 2012 and 2013. From 2014 all entrusted examination offices contribute to the database.

All examination offices committed themselves to continue their contribution to the the EU Potato DB. The follow up 2018 was added to the table for information.

		DE	PL	NL	UK	ES	IE	AT	CZ	SK	Total
Potato I	2006-2007	215	187	326	403						895*
NL/UK	2008-2011			220	184						404
Follow up	2012	46	33	39	32						150
Follow up	2013	23	38	37	22						120
Potato II	2014	49	36	47	21	2	9	11	15	5	195
Potato II	2015	44	17	57	11	0	6	6	9	3	153
Potato III	2016	35	19	56	5	0	8	6	12	2	143
Potato III	2017 Cand	30	15	40	7	0	4	6	14	7	123
Potato III	2017 CC	10	27	38	9	32	12	21	38	22	209
Ongoing	2018	26	26	45	6	0	5	0	8	0	116

\*) duplicates excluded

### 3.3 Review of the sampling procedure and DNA analysis

For the project DNA analysis was performed on two tubers per variety in two laboratories, SASA and Naktuinbouw. One laboratory has extracted and distributed the DNA. Each lab analyzed the DNA of one tuber. Results were crosschecked and agreed by the laboratories before reporting the results to the submitting examination office.

The aim of the project was also to improve the method in such a way that reliable descriptions can be produced in one lab.

#### 3.3.1 Lab experience

In 2004 CGN/PRI and SASA collaborated to produce from the public domain a harmonized set of 9 SSR markers yielding results that are both robust and easy to interpret. This set of 9 markers originated from 6 primer pairs developed in collaboration of SASA with the Scottish Crop Research Institute (SCRI), UK, and 6 SSR markers developed by the Centre for Genetic Resources (CGN) together with Plant Research International (PRI), NL. Later, Naktuinbouw was involved. The named marker system was used for the European Potato Database since 2006.

Experience of the labs is that reproducibility is generally higher when a system is set up by one lab and the testing is done by the same lab. Since this database is the result of a marker system that was developed by two labs, the most reliable results are obtained by analysis of both labs combined.

The possible impact of one vs. two labs was analyzed by the labs. For that purpose the labs investigated the number of discrepancies in allele scoring between the two labs during the period 2013 to 2017. Discrepancies can result from a number of factors. The relative contribution of each error type is indicated.

1. There is a new allele (for example the allele-bin for 2028 F actually contained 2 separate alleles now called F and K). These differences are normally resolved fairly easily by the labs agreeing on the presence of a new allele. – **3 %**
2. An allele was miscalled by one lab (can either be missed completely or assigned an incorrect letter). These are normally resolved fairly easily by both labs checking their data and agreeing on the correct call. – **44 %**



3. There is a genuine difference in the profiles obtained by the two labs. Fortunately a rare occurrence, usually the result of a sample with poor DNA quality that does not amplify well (2017/PL-006 was such a sample but between the two labs and two tubers it was possible to obtain a complete profile for this variety). Alternatively, this can be caused by a mixture of varieties, this is very easy to spot as the profiles are very different. (not counted for this analysis)
4. An allele is called as questionable by one lab (the lab cannot decide whether to call an allele or not) and IS called by the other lab. – **17 %**
5. An allele is called as questionable by one lab (the lab cannot decide whether to call an allele or not) and is NOT called by the other lab. – **36 %**

A considerable number of discrepancies were observed as shown in the following table. Better harmonization was reached since 2013 but there are still 20-25% of samples where clarification between the labs is necessary, at least for one marker.

Year	# samples	# discrepancies	discrepancy %
2013	121	74	61.2
2014	208	82	39.4
2015	156	32	20.5
2016	147	36	24.5
2017	121	27	22.3
Total	753	251	33.3

Discrepancies were not linked to specific markers or to specific alleles. Discrepancies of type 1, 2 and 3 are critical and have an effect on the calculation of the similarity values. Errors of type 4 and 5 are less critical. Both labs consider a type 4 discrepancy as not being a problem as the lab calling the allele as questionable thinks that there might be an allele present but it falls slightly below a predetermined threshold in the analysis software and the other lab definitely calls the allele as present. However, type 5 is more worrying as one lab thinks there might be an allele present but the other lab does not.

Both labs have developed decision rules. These are used as guidance in the interpretation and scoring of the alleles. Labs are aware from experience which alleles are more reliable on the ABI capillary system that is used by SASA and which of the alleles are always more clear to score on the LICOR gel-based system used by Naktuinbouw. There is not a better or more preferred machine/system. Both systems contribute equally to the accuracy of the DNA profiles.

With a sample size of two tubers only two admixtures were identified in the 753 samples submitted between 2013 and 2017.

The labs stated that cost for profiling did not change from 2013. They proposed to reduce cost by analyzing only one tuber instead of two. This would reduce the cost from 180 € to 150 € per sample. Cost for analysis of two tubers in the same lab was calculated with 150 € and for one tuber 90 €.

### 3.3.2 Service to be requested from laboratory

EOs have sent coded samples for DNA analysis to the labs for the basic service:

- DNA extraction
- PCR and electrophoresis with agreed method
- description
- delivery of descriptions to submitting EO

The following additional service was requested from the labs during the project:

- storage of profiles for analysis of similarities on request of EOs
- DNA storage
- research on specific DUS applications and improvement of the method

EOs are responsible for the management of molecular data in GEMMA.

The service to be requested from the lab(s) has to be reviewed regularly in conjunction with the service agreement (Annex 3 of Partnership agreement). It was agreed that the value of the European Potato Database would be increased if EOs would not only use the administrative and morphological data but also the molecular data for their own calculations. Therefore, project partners discussed the possibility to perform molecular similarity searches on their own platforms. It was agreed that it would not be appropriate to implement such search functions into GEMMA due to crop specific requirements and available separate software. GEMMA output files for molecular data will be adopted accordingly.

### **3.3.3 Conclusion**

The results of the labs were discussed during the second project meeting in January 2018. It was discussed whether it would be necessary to consider further risks in the process of DNA analysis, e. g. repeatability in the same lab, risk of miscalling in both labs, influence of DNA. The partners agreed that two tubers per variety are not important for uniformity assessment but for reliability of the DNA analysis, e.g. to identify possible errors. It was recalled that the profiling during the DUS test might be the only analysis of this variety over a long time and the result of this analysis is important for the EU Potato DB.

It was agreed that it is appropriate to continue with two tubers in two laboratories for the time being. The calculated potential cost savings do not justify the potential loss of reliability. The procedure will be reviewed regularly in conjunction with the service agreement (see Annex 3 of Partnership agreement).

It was suggested to consider a specific project to study potential risks in DNA profiling approaches. SSR markers in potato could serve as a model for such evaluations. Knowledge on the level of repeatability and reproducibility of the DNA methods and their impact on genetic similarity results is important for any construction and use of databases.

The service to be requested from the lab(s) has to be reviewed regularly

## **4. Management and continuation of the database**

The EU Potato Database is built up and maintained by the examination offices which are parties of the Partnership Agreement. The parties are committed to contribute with the agreed set of data gained in relation to DUS test of new potato varieties. Each party undertakes to use all reasonable efforts to regularly update the information in the database.

Further details for the contribution of data were defined in Annex 1 of the partnership agreement (see Annex 5).

### **4.1 Summary of data currently in the common EU Potato DB**

All partners have uploaded the agreed administrative data:

- Variety\_Id (numerical Id used by country)
- Denomination
- Breeder\_Reference
- Present\_Status
- Type of description

The following number of datasets was uploaded by 08/06/2018:

	AT	CZ	DE	ES	IE	NL	PL	SK	UK
Varieties	23	207	568	2	34	279	126	257	36
Phenotypic data CPVO-TP 023/2	13	184	312	2	34	240	103	203	29
Phenotypic data CPVO-TP 023/3			21						
Molecular data	13	37	164	2	17	197	46	9	35
No. of Photos (mostly 2 per variety)	23	37	144	2	58	412	0	228	0

## 4.2 Implementation of database into the DUS system

### 4.2.1 Molecular Similarity results

In addition to the profile of samples the submitting EOs received from the labs the result of the similarity analysis. Similarity information was transmitted for the variety pairs where the Jaccard similarity coefficient was 85 % or higher.

For clarification of possible questions on new applications partners exchanged information related to molecular data regularly.

All 100 % matches were expected or could be explained due to double submission of varieties from different EOs. Only 8 variety pairs were identified with a similarity of 85 % to < 100 %. The results and relevant follow up measures are summarized in the following table.

	2016		2017	
AT	6	no matches	6	no matches
CZ	12	no unexplained matches	14	no unexplained matches
DE	35	3 similarities (85 to <100%): clear morphol. differences	30	1 similarity (95.5%): comparison 2018; 1 similarity (86%): clear morphol. diff.
IE	8	no unexplained matches	4	no matches
NL	56	2 similarities (85 to <100%): 1 not maintained, 1 clear morphol. difference	40	no unexplained matches
PL	19	1 similarity (85 to <100%): not available for field comparison	15	no matches
SK	2	no matches	7	no matches
UK	5	no unexplained matches	7	no unexplained matches
	143		123	

ES – no candidates in 2016 and 2017

### 4.2.2 Country reports and ideas for future implementation

There are several ways of using the database in the DUS system of potato. EOs have developed ideas how the database can be incorporated in their systems in the near future (see Annex 4.1 to 4.9 Country reports).

It was noted that despite of the ringtests morphological variety descriptions from different countries are not sufficiently consistent. Own descriptions for the varieties are most important.

## **5. Partnership Agreements**

The agreement between partners as outlined in DOC-AC-2015-1-11 “CPVO R&D Strategy 2015-2020” was signed by all partners. By signing this agreement the partners commit themselves to cooperate in the database project beyond the duration of this action.

The specifications under which the parties shall contribute with data to the EU Potato DB forms Annex 1 to the Agreement. This annex 1 was agreed as attached in Annex 5 to this final report.

The CPVO has agreed with the labs on molecular analyses and with GEVES on the use of GEMMA for the duration of the project. As from 2018 on, terms and conditions for cooperation with the IT provider and with lab(s) have to be negotiated by the coordinator on behalf of the partners. Each partner will sign the ‘LICENSE AGREEMENT ON GEMMA Website & Database’ with GEVES. Currently contracts are finalized. For 2018, it was agreed to continue the cooperation with the labs under the same conditions as during the project. An agreement will be signed between the labs and the coordinator. Each partner will be charged by the labs directly for the submitted samples after providing all results according to the service agreement.

The contracts will be added as Annex 2 and Annex 3 to the Partnership Agreement after signature.

For the time being, it was not considered necessary to develop a separate Annex 4 on finances because all financial aspects are already covered in Annexes 1, 2 and 3. For the time being, no additional fee is foreseen for the use of the EU Potato DB and for its coordination.

In relation to Annex 5 on confidentiality of material it was agreed to use document DOC-AC-2015-2-13 – Annex 1 as adopted by the AC on 30/09/2015.

Annex 6 on confidentiality of data was agreed as attached in Annex 6 to this report.

## **6. Impact and benefits**

The EU Potato DB will include comprehensive up-to date information for all available varieties of the Common Catalogue and the candidate varieties applied for NLI or PBR in the EU (morphological and molecular data, lightsprout pictures, administrative information). The information is shared by all examination offices. The EU Potato DB and the close cooperation between the EOs will contribute to increase the quality of the DUS decisions and strengthening the NLI and PBR system.

The national reference collections including the in-house descriptions remain most important. But in general, these reference collections cannot include all varieties of common knowledge. In particular, the molecular marker information in the EU Potato DB provides an efficient tool to identify varieties which should be added to the national reference collection. Varieties not in the reference collection but with a high genetic similarity to a candidate variety can be added to the DUS growing trial. The risk to miss similar varieties will decrease.

Possible impact on the timeline of DUS tests depend on the national conditions. Currently, there is no or limited impact for most EOs as long as the molecular results are not available before planning the first growing trial (see Annexes 4.1 to 4.9).

Financial impacts will be handled on the national level. All EOs stated that the additional cost will be borne by the EOs in 2018. Considerations for the future are ongoing (see Annexes 4.1 to 4.9).

## **7. Conclusion and perspectives**

The EU Potato DB was successfully established comprising most varieties in the common catalogue. Mechanisms and rules for maintaining updated information were agreed and implemented. The additional efforts for maintenance and use of the DB will increase the quality of DUS decisions and will contribute to strengthen the system. Potential savings in the performance of growing trials depend on the national DUS systems. For the time being, most EOs do not expect fundamental changes in the field trials.

The establishment and maintenance of the EU Potato DB activated sustainably the cooperation between the EOs entrusted by the CPVO for DUS testing of potato. It can be considered as a model for a common DB. The experience in the project shows that cooperation between 10 partners requires continued coordinative work but it enables a better level of harmonization and exchange of information.

From 2018 onward, the EU Potato DB will be maintained, used and improved according to the conditions of the Partnership Agreement.

The following further actions have been considered:

- minor improvements linked to the GEMMA software
- download of molecular marker data and calculation of genetic distances by the EOs themselves
- proposal for a specific project to study potential risks and errors in DNA profiling approaches for the definition of quality requirements to be fulfilled by a lab

[Annexes follow]