CPVO · OCVV
Community Plant Variety Office
Office Communautaire des Variétés Végétales

CPVO IMODDUS STRATEGY PAPER

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Abbreviations used:

AC	*Administrative Council of the CPVO*
BMT	*Biochemical and Molecular techniques, and DNA Profiling in Particular (UPOV working group)*
CPVO	*Community Plant Variety Office*
CRISPR	*Clustered regularly interspaced short palindromic repeat*
DB	*Database*
DNA	*Deoxyribonucleic acid*
DUS	*Distinct, uniform, stable*
EDV	*Essentially derived variety*
GBS	*Genotyping by sequencing*
IMODDUS	*Integration of molecular data into DUS testing*
NBT	*New breeding techniques*
ODM	*Oligonucleotide directed mutagenesis*
PBR	*Plant breeders right*
SNP	*single-nucleotide polymorphism*
TALEN	*Transcription activator-like effector nucleases*
TGP	*Technical Guidance Protocol (UPOV document)*
TP	*Technical protocol*
UPOV	*International union for the protection of new varieties of plants*
VCU	*Value of cultivation and use*
WG	*Working Group*
WGS	*Whole genome sequencing*
ZNF	*Zinc-finger nucleases*

1. Introduction and background of CPVO IMODDUS

The CPVO has the firm conviction that biomolecular techniques could help to improve quality and efficiency in DUS testing and provide useful tools for the enforcement of plant breeders' rights. The creation of an ad hoc CPVO working group for the promotion of the use of bio-molecular techniques in DUS testing and variety identification was agreed by the CPVO Administrative Council (AC) in the framework of the adoption of the CPVO R&D Strategy for the period 2015-2020.

The name of the ad hoc working group is CPVO IMODDUS which stands for Integration of molecular data into DUS testing.

IMODDUS works as a think-tank on how to best integrate and promote molecular techniques into DUS testing. It assesses and discusses new developments in molecular techniques and their potential or immediate use for DUS of certain species, variety identification and enforcement. It should identify relevant species where these techniques would be promising; to harmonize methodologies and to promote and propose R&D projects for co-funding by the CPVO. A limited number of designated experts from entrusted Examination Offices, experts from the breeding industry appointed by the breeders' organizations and experts from research institutions, universities or other testing centers are invited to attend the IMODDUS meetings due to their specific knowledge in matters related to molecular techniques. Representatives from the Commission (DG SANTE) and, on request, a few experts from the United States Department of Agriculture can participate via audio conference.

2. The framework on the use of biomolecular techniques in DUS testing

The below described framework should in no way be understood as a fixed frame work but should be used as a basis for coming discussions in IMODDUS meetings. In its function as a Think-Tank, IMODDUS is explicitly invited to explore new models and concepts even though they may not fall within the present legal frame work. As in many other sectors, technical developments evolves before existing legal frameworks.

EU Legal frame work

The following legal considerations are of general nature since any new method would need to be analysed on a case by case basis.

The CPVO framework for conducting DUS testing is the Basic Regulation, the Implementing Rules, the CPVO Technical Protocols and guidance documents adopted by UPOV. When carrying out a technical examination Article 56 of Regulation 2100/94 on Community Plant Variety Rights states the following: 'Unless a different manner of technical examination relating to compliance with the conditions laid down in Articles 7 to 9 [DUS] has been arranged, the Examination Offices shall, for the purposes of the technical examination, grow the variety or undertake any other investigations. The conduct of any technical examination shall be in accordance with the test guidelines issued by the Administrative Council and any instructions given by the Office. So it seems that under the applicable legal frame work molecular techniques could be used as complementary tool.

As in the EU the same CPVO/UPOV test protocols are used both for variety protection and registration there is a need to analyse the Directives on Common Catalogues (Council Directives 2002/53/EC and 2002/55/EC) as well. According to Article 7(1) the acceptance of varieties be based on the results of official examinations, particularly growing trials, covering a sufficient number of characteristics for the variety to be described. The requirement on growing trials is strengthened by the fact that for the establishment of distinctness, the growing trials shall include at least available comparable varieties (Article 7(1)). So it seems that under the applicable legal frame work growing trials cannot be replaced and that molecular techniques could only be used as complimentary tool in addition to the growing trials.

Considerations in relation to the UPOV frame work

On international level, the agreed framework of UPOV Members as regards the application of the use of molecular techniques in DUS testing is laid down in document UPOV/INF/18, it has been adopted by the Council of UPOV in 2011.

The purpose of document UPOV/INF/18 is to provide guidance on the use of molecular techniques in DUS testing and has elaborated the conditions for it. The two conditions for the application of molecular techniques are that its application should be in conformity of the UPOV Convention and that it should not undermine the strength of the protection of a variety.

UPOV/INF/18 displays different models, described by concrete examples, and whether they had received a positive or a negative assessment as regards their conformity with the UPOV convention. It further leaves room for the development of new models, subject to the above mentioned assessment by the relevant UPOV bodies.

This paper will elaborate on the positively assessed models by describing their concepts and on new concepts under an approved model which are currently under discussion in the UPOV-BMT working group. The paper will also address potentially new models.

Although the EU and most of its Member States are UPOV members, discussions that go beyond what is traditionally seen as comprised by the present wording of the UPOV Convention may take place in IMODDUS. The UPOV Convention has been in force for more than 50 years and has proven to be an excellent tool for stimulating innovation in the plant sector. However, techniques are developing rapidly and it is important that the EU looks forward and embarks on a strategy which is adapted to test plant varieties by using new technologies.

Conclusions of Imoddus

The purpose of this document is to gather thoughts and ideas in one document to ease further discussions in IMODDUS. The CPVO AC is informed on the contents of this paper but the intention is not that it needs to be adopted by the AC. If conclusions are drawn by IMODDUS which could potentially be translated into new methods or procedures to be used in variety testing, the CPVO will make proposals to the AC for discussion and adoption.

3. Models and concepts

For the purpose of this document the wording model refers to the meaning as described in UPOV/INF/18 whereas "concept" refers to a concrete example of an application within that model.

3.1. UPOV-accepted model 1: Characteristic specific markers

Molecular characteristics can be used as a predictor of traditional characteristics if a clear link exists. The example given was an herbicide resistance which had been introduced and of which its existence could be proved by the use of the relevant marker.

IMODDUS fully support this model and encourages Examination Offices to use it. However, IMODDUS should not concentrate on discussing such an accepted model for which applicable concepts have already proven to work.

3.2. UPOV-accepted model 2: Concepts for the management of reference collections

3.2.1. Combining molecular and phenotypic thresholds

Two independent thresholds are set for the selection of similar varieties to be included into the growing trial:
The first threshold is based on the information of morphological characteristics and the second threshold is based on the genetic distance of a set of markers by calculating e.g. Roger's distance or Jaccard's coefficient. Except

for morphologically very similar varieties, reference varieties exceeding the two thresholds do not need to be included into the growing trial.

This concept is approved and already under application for species such as maize, lettuce, barley and others. It has shown to reduce significantly the number of pairwise comparisons in the DUS trials.

If this exercise is conducted only after the first growing cycle had been finalised, using the morphological description of the candidate variety obtained from that first growing cycle, this approach has some similarity to the concept described hereunder in point 3.2.2

IMODDUS fully support this model and encourages Examination Offices to use it. However, IMODDUS should not concentrate on discussing such an accepted model for which applicable concepts have already proven to work.

3.2.2. Genetic first selection of similar varieties for the growing trial

This concept has been presented by the Netherlands at the BMT/15. The naming of the concept is a proposal provided by the Netherlands. A concrete example for the management of the reference collection of a seed propagated species with more than one growing cycle, condition for the application of that model, is available in document BMT/15/21.

The candidate variety is DNA profiled against a defined set of markers, the genetic distance to the varieties from the reference collection is calculated. In that concrete example, all reference varieties with a genetic similarity higher than 80% are to be grown with the candidate variety in the first growing cycle in which the candidate variety is assessed on uniformity and described morphologically, according to the technical protocol. The variety descriptions used have been setup based on observations made at that testing station (in-house variety descriptions). If variety descriptions used which are not made in-house, those must be sufficiently robust through harmonization exercises with other examination offices such as ringtests. This description is checked against the variety descriptions of reference varieties and those which are identified to be similar will be included for comparison into the second growing trial. In case the variety was clearly distinct from the similar varieties in the first growing cycle and no similar varieties have been detected based on the variety description after the first growing cycle, a positive decision on distinctness could be taken after one growing cycle.

The BMT agreed that this concept should be taken up into TGP/15 as a new example for the accepted UPOV model 2. For that purpose the concept needs to elaborate further about the conditions as regards the quality of the variety descriptions. In addition, the concept needs to elaborate on how to overcome the time constraints relating to the moment of the reception of plant material and the subsequent molecular analysis of the samples.

As for any new model, it is necessary to gain more experience as regards the definition of marker sets, criteria for the markers to be chosen, and reliable, adapted genetic distance thresholds for that concept. In that light IMODDUS could concentrate on developing this concept.

3.3. New models under discussion within UPOV-BMT but not (yet) approved

At BMT/14 and BMT/15 several concepts had been presented referring to the calculation of genetic distances. Those applications, described hereunder, would not apply for the purpose of establishing distinctness while using genetic distances as characteristics. For that reason, it is considered that these concepts would fall under a new model.

3.3.1. "American model" of genetic distances to reference varieties

An approach under investigation in the United States of America for potential application in Plant Variety Protection is the use of reference varieties in varietal distinctness. It is described in detail in document BMT/14/5 and BMT/14/5 Add and had been presented by ASTA on behalf of ISF.

The concept is to utilize the genetic relationship of a candidate variety to a set of known, pre-determined "reference" varieties. Similar to the GPS system where a location can be determined by its distance to a number of reference Cities, genetic similarity coefficients with a known set of reference varieties can be used to distinguish between genotypes. Each reference variety would be treated as a new characteristic. The primary tool for establishing distinctness would remain to be the morphological characteristics but if necessary, the genetic distance characteristics could be added to the technical protocol and as such be displayed in the variety description.

ASTA stated that the proposal was at an early stage of consideration by the Plant Variety Protection Office in the United States of America. The BMT had agreed that it would be useful to invite the Technical Committee to consider the possible approach for the use of molecular markers.

This concept opens new possibilities to establish distinctness. The feasibility of this concept would need to be tested for different types of species (autogameous/allogameous, definition of reference varieties, maintenance of distance etc.). In that light IMODDUS could cooperate on developing this concept. Enhanced cooperation with the US experts should be explored.

3.3.2. Transformation of genetic distances into characteristics of the TP

This concept has been presented by the Netherlands at the BMT/15 with document BMT/15/22 for discussion. It is based on the above "American model" and develops the idea further as to whether the genetic distance to reference varieties could be transformed into characteristics with states of expressions to be used for the establishment of D and the description of the variety.

The example was given on the species *Phalaenopsis*, a vegetatively propagated species with a huge number of varieties of common knowledge. The Jaccard distance to each of the three reference varieties was transformed into 9 states of expression by using equally spaced notes. That meant that for a candidate variety the difference in genetic distance to three pre-defined reference variety was expressed in notes. It was suggested that since the distance had been transformed into notes, it would be possible to apply the same rules as for notes obtained for morphological characteristics. The definition and choice of the set of markers to be used is an important step in the whole procedure.

The BMT had agreed that more work should be done on this approach. It furthermore had agreed that it could be useful to consider a possible use of this specific type of characteristic comparable to the approach used for isoenzymes or storage proteins in the Test Guidelines for certain cereal species.

Strategy: This concept builds up on the American model. Issues to be answered are whether the transformation into states of expressions makes this new type of characteristic usable according to the UPOV rules. The "status" of this new type of characteristics need to be clarified, use of this tool as "supporting evidence" only or on regular basis for each DUS test.

In order to evaluate their potential use for DUS testing, the principles should be tested in concrete projects on species which are deemed to be suitable for that purpose and could serve as a pilot species. In that light IMODDUS could cooperate on developing this concept.

3.3.3. New models based on molecular techniques

Huge progress has been made since the first development of molecular techniques. The latest developments are Whole Genome Sequencing (WGS) or Genotyping by Sequencing (GBS). In addition, these new marker techniques have evolved to become much cheaper. Given this environment, many possibilities are open for the exploration of the application of molecular techniques.

A completely new way to proceed would be to reverse the use of new and traditional methods. A scenario could be explored whereby tests would start with by a step using molecular/DNA techniques and then use the current, phenotypical, approach only in case of need. Such an approach would certainly impact and challenge the present system and may have consequences which are not easy to establish at a first look. For this reason, IMODDUS should elaborate on the impact such a new model may have on procedures and ultimately the decisions taken concerning Plant variety rights, the registration of varieties and the certification of plant material. The paper could include technical as well as legal considerations.

It was agreed in the Imoddus meeting in January that the CPVO will draft a paper on such a model to be discussed electronically amongst the participants. Such a model and potential consequences of using it will then be added to the Strategy paper.

3.4. Handling of "Big Data"

The new molecular techniques create enormous amounts of data and require expertise on the interpretation of these data. While the price of sequencing a genome decrease, costs to build IT-structures and compute such information increase. Analysis done by single computers and offices will become difficult to execute.

Adding an agenda point covering this issue will reserve room for that important aspect of introducing new techniques into DUS testing. Points of discussion to be addressed are

- Building capacities on analyzing big data for the purpose of DUS tests
- IT requirements on big data and how to meet them
- IT security and confidentiality aspects

Strategy: Address issue of big data in agenda and get inspiration from existing Research structures in the European Union and analyze tools to benefit from their experience. Keep in mind to enhance collaboration between Bio-informaticians and experts of DUS and of molecular techniques.

3.5. New breeding techniques (NBT) and molecular techniques for the purpose of DUS testing

A number of NBT have been developed and are applied more and more often to create new plant varieties. These are, amongst others, clustered regularly interspaced short palindromic repeat (CRISPR), oligonucleotide directed mutagenesis (ODM), transcription activator-like effector nucleases (TALEN), or zinc-finger nucleases (ZNF).

In the classical DUS test, the way a variety has been bred/developed has no impact on the way the DUS test is conducted. Only the way of multiplication impacts on the DUS test, e.g. uniformity is assessed in a different manner for autogameous species than for allogameous species. This counts as well for single hybrids compared to three-way-hybrids or fruit varieties obtained through mutation compared to varieties obtained by crossing and seedlings.

The way of multiplication impacts as well the number of plants to be observed in the trial. But beyond the uniformity assessment, the breeding method has no impact on the assessment of distinctness.

In that sense, the fact of using NBT for the creation of new varieties does in principle not imply a change of the DUS test. It might be necessary that additional characteristics need to be observed, depending on the features the new variety displays, or on request of the breeder for a specific feature to be observed as single difference to a parent variety. This can be done as today, by classical observation of the phenotype, and/or by using molecular techniques, under the condition that the relevant sequence is known and can be targeted for its presence/absence expression by these techniques.

From today's perspective it is expected that the number of varieties being developed with the use of advanced molecular techniques will increase. However, as stated by the BMT in May 2016, it is likely that the impact on the DUS test remains limited but may become more an issue for breeders as it probably increases the number of essentially derived varieties.

Add NBT to the agenda of IMODDUS in order to make sure that latest developments are addressed at in the meeting.

4. Enhance developments of molecular techniques and its use in DUS

In order to stimulate the integration of molecular techniques into DUS testing it is of great relevance to get scientific input, to identify the objectives together with the relevant stakeholders, to organize the collaboration, also with other internationally working bodies, and to make available the necessary funding.

4.1. Measures to get scientific input

It is important to get input from scientific developments in order to assess research results on their potential use for implementation into DUS testing. The UPOV-BMT meeting which take place every 18 months is an excellent platform for this purpose.

In addition, means need to be put in place to be able to closely follow scientific publications. That could be achieved by commissioning such a task to Scientists on an agreement level with the CPVO.
Such knowledge should materialize into R&D project proposals which would be discussed in the relevant circles.

It is important to gather the input from breeders on their use of molecular techniques as a tool used for the creation of new varieties. Since breeders participate to all crop expert meetings organised by the CPVO, the issue should be placed on the agenda of these meetings.

IMODDUS meetings shall foresee at least half a day of presentations given by Scientists.

4.2. International cooperation

Developments in molecular techniques are also ongoing in other bodies dealing with plant varieties and it is necessary to make collaboration happen, as described and agreed upon between UPOV, OECD and ISTA.

IMODDUS should keep this in mind and observe international meetings and contribute where possible. It should also follow closely the developments in order to evaluate potentially new methods on their usefulness as an internationally harmonized method for variety testing.

Collaborate with international authorities dealing with variety testing and identification.

4.3. Funding

To carry out R&D projects require money and time. So far, CPVO co-funded projects together with examination offices, and in some cases with Breeders associations.

The CPVO budget for R&D foresees € 500.000 for 2017. A decision on the budget is taken by the Administrative Council of the CPVO. Although this budget line may be increased in the future it is not foreseen that a high part of the CPVO budget should be devoted to R&D.

The financial situation at the Examination Offices seem to be very different, some invest regularly into projects focused on molecular techniques but the majority seem not to be involved in this type of work with own, associated laboratories.

Recent project proposals discussed in IMODDUS were designed to essentially collect data for the management of big reference collections. Those are to be used with the aim to limit the number of varieties grown in the field test and hence to contribute to cost efficiency. The proposal was to ask for the financial participation of the Breeders for this type of variety characterization of reference collections. That option should be kept in mind when such type of project is under discussion. The underlying principle is cost efficiency and such projects should be self-financed at some point.

The European Commission makes available the Research and Development Budget under the name "Horizon 2020". CPVO, in collaboration with interested examination offices, should investigate on the possibility to get funding from this source. This might be done with the help of specialized agencies.

It is proposed that "Funding" will be taken up on the agenda of meetings of IMODDUS. Action should be taken to explore how funds can be made available from research programs such as, e.g. "Horizon 2020". Identify projects on a case by case basis which could be co-funded by the Breeders' associations.

5. Environment to implement the strategy

5.1. IMODDUS meetings

CPVO is responsible for the organization of the IMODDUS meetings. The composition of the group is determined by the CPVO depending on the needs. Experts receive personal invitations to attend the meeting. That meeting should address the issues as mentioned in this strategy paper.

5.2. Role of IMODDUS in CPVO R&D procedure

As far as concrete R&D project proposals are concerned, the opinions gathered from the IMODDUS working group should be taken into account by the CPVO when it sets up a list of priority in case several proposals have been received. R&D proposals which are retained by the CPVO will be subject to consultation by the relevant crop experts group and the CPVO R&D Advisory group. Based on the assessment of the project proposal by the CPVO R&D Advisory group the President of the CPVO will take a final decision on the granting of the project or not.

5.3. Collaboration with CPVO network of Examination Offices and Breeders

Discussions on R&D projects need to make sure that DUS experts and Breeders are involved as early as possible. Experts' knowledge shall help to identify the 'right' species to test in a project the feasibilities of a given model/concept. In addition, they are best placed to know where problems in DUS testing exist.

The agenda for the CPVO crop experts meetings mentions always "R&D projects" on the relevant agenda. Feedback from IMODDUS discussions is given at the relevant crop experts meetings.

5.4. Cooperation between laboratories

It is our understanding that so far four examination offices (ES, FR, NL and UK) have laboratories where molecular techniques are frequently used for modern genetic analysis of plants and seeds. In addition, EU's Joint Research Center (JRC) has both in Ispra/Italy and in Geel/Belgium such laboratories and JRC has ample experience with bio-molecular analyses and validation of methods.

To equip a laboratory with the state of art equipment and highly specialized human resources requires investments that are not negligible. It is for that reason questionable if the best way forward would be to require entrusted examination offices in all 28 Member States to make such investments.

It may be better value for money that a limited number of laboratories will work on behalf of the CPVO for the purpose of assisting in the carrying out of technical examinations and that those laboratories can also provide services to other examination offices. This is of course a political issue that each Member State will need to take a position on but it is nevertheless worthwhile taking it up in this paper which addresses the use of molecular techniques for the future and how the CPVO should manage the use of such techniques.

The CPVO believes that it is worthwhile exploring the possibility of creating a network of laboratories involved in this work. This way the methods used can be harmonized to ensure that results are comparable, for instance, which techniques should be used (SNP etc.). It could also be elaborated and discussed how genetic analysis could be done within/between CPVO and entrusted examination offices. In essence, how can a solid and user friendly system within the EU be developed at reasonable costs?

Identification of the types of analyses and the approach to be followed (the two steps can be separated or done in one lab):

1. Preparation of the DNA material and analysis of the DNA (each entrusted EO, EO with laboratory, external provider, breeder)
2. Interpretation of the DNA results (each entrusted EO, EO with laboratory or somewhere centralized (maybe by species), or as much as possible automatized analysis)).

IMODDUS should investigate on the organisation of a network with a limited number of laboratories and on the quality and the status of such laboratories in relation to the CPVO network of entrusted examination offices. Accreditation possibilities should be explored as well as means for the standardization and validation of methods.

5.5. Collaboration with Breeders

Any developments on the use of molecular techniques in DUS includes discussions with the breeders Community. Furthermore it is important to take into consideration their position papers (ESA, ISF, CIOPORA and Plantum). Exchange of views is guaranteed today by their regular participation to the CPVO crop experts meetings and also to UPOV working party meetings. In addition, the CPVO has regular bilateral meetings where matters relating to molecular techniques should be added to the agenda, if deemed necessary.

Breeders associations are invited to participate to the IMODDUS meetings, to the CPVO crop experts' meetings and have bilateral meetings with the CPVO.

6. Implementation of successful models/concepts into DUS testing and output

Under the assumption that some projects deliver successful results, the implementation into DUS testing must be organized. In case a new model or concept has been successfully tested, it would need to be validated, a standardized method through an inter-laboratory comparison at international level and a new model/concept by the relevant UPOV bodies.

For already approved models and concepts, it must be checked whether the new technique needs to be taken up into the relevant CPVO-Technical Protocol or not. If this concerns a specific characteristic, that is evident, however, for example the management of the reference collection is not mentioned in detail in the CPVO-TP, instead it makes reference to the relevant UPOV-TGP document. A result could thus be that a revision of such TGP document on UPOV level is requested.

The same exercise needs to be conducted as regards a potential adaptation of the CPVO quality requirements and the CPVO Designation Agreement with Examination Offices.

Depending on the project, stakeholders need to assess if further implementation is preferable, e.g. to amend variety descriptions etc.

7. Roadmap and reporting

This CPVO strategy paper is the result of discussions with IMODDUS experts. A final draft will be submitted to the Administrative Council of the CPVO for discussions.

IMODDUS reports to the CPVO crop experts groups, the CPVO Administrative Council and to the relevant UPOV bodies. Breeders associations participate to these meetings but CPVO can report at the occasion of the bilateral meetings.

IMODDUS meetings should take place once per year and, depending on the agenda, take between one or two days.