UPOV

International Union for the Protection of New Varieties of Plants

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Technical Committee **TC/55/7 Add. 2**

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SECOND ADDENDUM TO MOLECULAR TECHNIQUES

Document prepared by the Office of the Union

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EXECUTIVE SUMMARY

1. The purpose of this second addendum is to report on developments on the use of biochemical and molecular techniques in DUS examination at the thirty-seventh session of the Technical Working Party on Automation and Computer Programs (TWC) and at the eighteenth session of the Working Group on Biochemical and Molecular Techniques, and DNA-Profiling in Particular (BMT).

2. The structure of this document is as follows:

COOPERATION BETWEEN INTERNATIONAL ORGANIZATIONS

Inventory on the use of molecular marker techniques, by crop

3. The Technical Working Party on Automation and Computer Programs (TWC), at its thirty-seventh session, held in Hangzhou, China, from October 14 to 16, 2019, and the BMT, at its eighteenth session, considered the following elements for the inventory on the use of molecular marker techniques, by crop, which had been developed in consultation with the OECD, as set out in document TWP/3/7 "Molecular techniques", paragraph 81 and in document BMT/18/4, paragraph 25:

Country or Intergovernmental Organization using molecular marker technique
Source [the name of the Authority] and Contact details [email address]
Type of molecular marker technique
Crop (s) for which the molecular marker technique is used [botanical name(s) and UPOV code(s) to be provided]
Purpose of the use of the molecular technique [UPOV model "Characteristic-Specific Molecular Markers", UPOV model "Combining Phenotypic and Molecular Distances in the Management of Variety Collections", Purity, Identity, Verification of hybridity]
Is the molecular marker technique used as part of Seed Certification in the last two years? [National certification, OECD certification] [relevant for OECD seed schemes]
In the last 2 years, how many times did the Authority use the molecular marker techniques?
The molecular marker technique is covered by [UPOV Test Guideline(s), UPOV TGP document(s), other document(s) (please specify)]
Is the molecular technique validated? [If yes, please specify a particular organization or authority] [relevant for OECD seed schemes]

4. The TWC endorsed the elements above for the inventory on the use of molecular maker techniques, by crop (see document TWC/37/12 “Report”, paragraph 80).

5. The BMT agreed that the survey should structure answers to allow the comparison of results. For example, the question “Type of molecular marker technique” should provide a list of possible answers (see document BMT/18/21 “Report”, paragraphs 25 to 31).

6. The BMT agreed to propose the addition of the following initial question: “Does your Authority use molecular marker techniques?”

7. The BMT agreed with the TWA that the question “Is the molecular technique validated?” should not be included in the survey.

8. The BMT agreed that the survey should allow information to be provided on the use of more than one molecular marker technique per crop (branching structure at crop level).

9. The BMT agreed with the TWA that the question “In the last 2 years, how many times did the Authority use the molecular marker techniques?” should be clarified to explain whether the value provided referred to routine or exceptional use of the technique (e.g. screening of variety collections). The BMT agreed that this question should have structured answers with ranges of values (e.g. “1 to 5”; “6 to 20”; “21 to 100”).

10. The BMT agreed with the proposal by the TWA to add a question on whether respondents had established databases with information obtained from the molecular markers used.

11. The BMT agreed that a test survey should be considered before inviting members to respond.

Lists of possible joint initiatives with OECD and ISTA in relation to molecular techniques

12. In response to the request to develop lists of possible joint initiatives with OECD and ISTA, in relation to molecular techniques, the BMT, at its eighteenth session, agreed to propose the repeating of joint workshops with ISTA and OECD in future. The BMT agreed to propose a joint initiative that each organization inform the others about use of molecular markers in their work (see document BMT/18/21 “Report”, paragraph 34).

Joint document explaining the principal features of the systems of OECD, UPOV and ISTA

13. The BMT, at its eighteenth session, held in Hangzhou, China, from October 16 to 18, 2019, considered document BMT/18/4 “Cooperation between International Organizations” and agreed that relevant elements from the World Seed Partnership and the FAQ on the use of molecular techniques in the examination of DUS, as reproduced below, would be a suitable basis for the Office of the Union to develop a draft of a joint document explaining the principal features of the systems of OECD, UPOV and ISTA, in consultation with OECD (see document BMT/18/21 “Report”, paragraphs 22 and 23).

Relevant elements from the World Seed Partnership

> What is the World Seed Partnership?
>
> The World Seed Partnership is host to a group of international organizations that closely collaborate on seed systems for sustainable agriculture. Below are short summaries and full profiles of participating organizations.
>
> The Organisation for Economic Co-operation and Development (OECD)
>
> Type of Organization
> intergovernmental
>
> OECD Seed Schemes
> Participating countries
>
> Mission
> The OECD Seed Schemes provide an international framework for the varietal certification of agricultural seed moving in international trade. The Schemes were established in 1958 driven by a combination of factors including a fast growing seed trade, regulatory harmonisation in Europe, the development of off season production, the seed breeding and production potential of large exporting countries in America (North and

South) and Europe, and the support of private industry. Membership of the Schemes is voluntary and participation varies. There are eight agricultural Seed Schemes.

Objectives

- to encourage the production and use of “quality-guaranteed” seed in participating countries. The Schemes authorise the use of labels and certificates for seed produced and processed for international trade according to agreed principles ensuring varietal identity and purity.
- to facilitate the import and export of seed, by the removal of technical barriers to trade by assuring identification and origin through internationally recognised labels (“passports”) for trade. They also lay down guidelines for seed multiplication abroad, as well as for the delegation of some control activities to the private sector (“authorisation”). The quantity of seed certified through the OECD Schemes has grown rapidly in recent years and now exceeds 1 million tonnes.

How do the Seed Schemes operate
The success of international certification depends upon close co-operation between maintainers, seed producers, traders and the designated authority (appointed by the government) in each participating country. Frequent meetings allow for a multi-stakeholder dialogue to exchange information, discuss case studies, revise rules and update the Schemes. A wide range of international and non-governmental organisations as well as and seed industry networks participate actively in the Schemes.

Benefits of the Schemes

- To facilitate international trade by using harmonised certification procedures, crop inspection techniques and use of control plots. The varietal purity standards for the appropriate species are also agreed and standardised by all member states.
- To provide a framework to develop seed production with other countries or companies.
- To participate in the elaboration of international rules for seed certification.
- To develop collaboration between the public and private sectors.
- To benefit from regular exchanges of information with other national certification agencies and Observer organisations.

The Annual List of Varieties eligible for OECD certification includes varieties which are officially recognized as distinct, uniform and stable, and possess an acceptable value in one or more participating country. The List contains the seed varieties internationally traded using the OECD seed Schemes. The number of varieties included has grown steadily over the last thirty years.

International Union for the Protection of New Varieties of Plants (UPOV)

Type of Organization
Intergovernmental

Membership
List of UPOV members / Situation in UPOV

What is UPOV?
The International Union for the Protection of New Varieties of Plants (UPOV) is an intergovernmental organization based in Geneva, Switzerland. UPOV was established in 1961 by the International Convention for the Protection of New Varieties of Plants (the "UPOV Convention").
The mission of UPOV is to provide and promote an effective system of plant variety protection, with the aim of encouraging the development of new varieties of plants, for the benefit of society.
The UPOV Convention provides the basis for members to encourage plant breeding by granting breeders of new plant varieties an intellectual property right: the breeder’s right.

What does UPOV do?
UPOV’s mission is to provide and promote an effective system of plant variety protection, with the aim of encouraging the development of new varieties of plants, for the benefit of society. The main objectives of UPOV are, in accordance with the UPOV Convention, to:

- provide and develop the legal, administrative and technical basis for international cooperation in plant variety protection;
- assist States and organizations in the development of legislation and the implementation of an effective plant variety protection system; and
- enhance public awareness and understanding of the UPOV system of plant variety protection.

What are the benefits of plant variety protection and UPOV membership?

The UPOV Report on the Impact of Plant Variety Protection demonstrated that in order to enjoy the full benefits which plant variety protection is able to generate, both implementation of the UPOV Convention and membership of UPOV are important. The introduction of the UPOV system of plant variety protection and UPOV membership were found to be associated with:
(a) increased breeding activities,
(b) greater availability of improved varieties,
(c) increased number of new varieties,
(d) diversification of types of breeders (e.g. private breeders, researchers),
(e) increased number of foreign new varieties,
(f) encouraging the development of a new industry competitiveness on foreign markets, and
(g) improved access to foreign plant varieties and enhanced domestic breeding programs.
In order to become a UPOV member the advice of the UPOV Council in respect of the conformity of the law of a future member with the provisions of the UPOV Convention is required. This procedure leads, in itself, to a high degree of harmony in those laws, thus facilitating cooperation between members in the implementation of the system.

International Seed Testing Association (ISTA)

Type of Organization
Non-profit and non-political association

ISTA Profile
ISTA is an international association that represents the seed quality sampling and testing organizations and laboratories at the world level.

ISTA Members
List of ISTA Members

Mission
ISTA was founded in 1924 with the aim of developing and publishing standard procedures in the field of seed testing. ISTA members work together to achieve their vision of uniformity in seed quality evaluation worldwide.

Core tasks

1. Development and maintenance of the ISTA International Rules for Seed Testing
   The International Rules for Seed Testing (ISTA Rules), adopted and updated on an annual basis, today contain seed sampling and quality analysis methodologies for more than 900 different agricultural, forest, vegetable and flower species. The ISTA Rules are reviewed and updated on an annual basis by 18 technical committees. The technical committees comprise seed scientists and technologists from the public and the private sectors from all over the world.
2. Accreditation of seed testing laboratories worldwide
   The ISTA accreditation program ensures that seed testing laboratories achieve accurate and reproducible results in their daily analysis work. The basis for the accreditation programme is the ISTA Accreditation Standard. Every third year, an accredited laboratory is audited by two ISTA auditors. Monitoring of laboratory performance through the ISTA Proficiency Test Programme ensures that the quality of ISTA-accredited laboratories remains high between audits. Each year between five and ten workshops, run by the technical committees, provide training and professional development for seed analysts.
3. Distribution of uniform certificates of seed-testing results to facilitate international seed trade
   Only ISTA-accredited laboratories are authorized to issue ISTA Certificates for seed analysis. The ISTA certificates provide the user with a seed analysis result they can trust is reproducible, true and, and for the Orange International Seed Lot Certificate represents the quality of the seed lot from which the sample tested was drawn.
4. Exchange and dissemination of results of scientific research in various seed symposia, seminars and scientific journals
   ISTA serves as a platform for seed scientists around the world to compare the results of their research and discuss important developments in seed science and technology, through both regular seed symposia and its own scientific journal, Seed Science and Technology.

International Seed Federation (ISF)

What is ISF?
ISF is a non-governmental, non-profit making organization that represents the interests of national seed associations and seed companies at a global level. Established in 1924, the International Seed Federation has more than 7500 members in 70 countries today. Working in partnership with organizations responsible for international treaties, conventions and agreements and those that shape policies that impact the seed industry, ISF ensures that the seed industry speaks with one voice.

Vision & Mission

- Vision: “A world where the best quality seed is accessible to all, supporting sustainable agriculture and food security.”
- Mission: “To create the best environment for the global movement of seed and promote plant breeding and innovation in seed.”

Objectives
ISF’s strategic objectives are set out in its 5-year Strategic Plan, and relate to the core areas of its work.

1. Innovation
To move towards more consistent policies for products developed through the latest plant breeding methods to enable their use and to ensure uninterrupted trade.
2. Movement of Seed & Quality Seed
   - To promote the harmonization of technically and scientifically justified frameworks for phytosanitary measures and to prevent them becoming non-tariff trade barriers.
   - To promote the harmonization of regulations governing seed applied technologies at global and regional levels.
   - To promote the use of seed certification schemes and seed quality assurance systems.
3. Intellectual Property Rights
   - To facilitate cooperation between countries in order to simplify procedures for plant variety protection at an international level.
   - To support members in implementing effective intellectual property rights in their countries.
   - To promote the International Treaty as the preferred tool to administer Plant Genetic Resources for Food and Agriculture (PGRFA), making the process more business-oriented and user-friendly.

4. Biodiversity
   - To promote the International Treaty as the preferred tool to administer Plant Genetic Resources for Food and Agriculture (PGRFA), making the process more business-oriented and user-friendly.
5. Engagement
   - To engage with our members to strengthen cooperation so that the seed industry speaks with one voice.
   - To engage with all stakeholders in the value chain to foster cooperation.
   - To raise awareness and build understanding of the seed industry and the benefits it brings to a global society.

What does ISF do?

- ISF facilitates the free movement of seed within a framework of fair and science-based regulations, whilst serving the interests of farmers, growers, industry and consumers.
- ISF promotes the establishment and protection of intellectual property rights for seeds, plant varieties and associated technologies.
- ISF publishes rules for trading seed and licensing technology to clarify and standardize contractual relations between buyers and sellers at an international level.
- ISF provides for the settlement of disputes through mediation, conciliation and/or arbitration.
- ISF fosters cooperation and collaboration through its calendar of events, enabling seed industry stakeholders to identify issues, stimulate strategic thinking and accelerate the adoption of common positions.
- ISF works in partnership with organizations responsible for international treaties, conventions and agreements and those that shape the policies affecting the global seed industry.

Source: http://www.worldseedpartnership.org/

FAQ on the use of molecular techniques in the examination of DUS

Does UPOV allow molecular techniques (DNA profiles) in the examination of Distinctness, Uniformity and Stability (“DUS”)?

It is important to note that, in some cases, varieties may have a different DNA profile but be phenotypically identical, whilst, in other cases, varieties which have a large phenotypic difference may have the same DNA profile for a particular set of molecular markers (e.g. some mutations).

In relation to the use of molecular markers that are not related to phenotypic differences, the concern is that it might be possible to use a limitless number of markers to find differences between varieties at the genetic level that are not reflected in phenotypic characteristics.

On the above basis, UPOV has agreed the following uses of molecular markers in relation to DUS examination:

(a) Molecular markers can be used as a method of examining DUS characteristics that satisfy the criteria for characteristics set out in the General Introduction if there is a reliable link between the marker and the characteristic.

(b) A combination of phenotypic differences and molecular distances can be used to improve the selection of varieties to be compared in the growing trial if the molecular distances are sufficiently related to phenotypic differences and the method does not create an increased risk of not selecting a variety in the variety collection which should be compared to candidate varieties in the DUS growing trial.

The situation in UPOV is explained in documents TGP/15 “Guidance on the Use of Biochemical and Molecular Markers in the Examination of Distinctness, Uniformity and Stability (DUS)” and UPOV/INF/18 “Possible use of Molecular Markers in the Examination of Distinctness, Uniformity and Stability (DUS)”.

https://www.upov.int/about/en/faq.html#QB80

SESSION TO FACILITATE COOPERATION IN RELATION TO THE USE OF MOLECULAR TECHNIQUES

Developments at the thirty-seventh session of the Technical Working Party on Automation and Computer Programs

14. The TWC, at its thirty-seventh session, considered document TWP/3/7 “Molecular Techniques” and formed discussion groups to allow participants to exchange information on their work on biochemical and molecular techniques and explore areas for cooperation. The following information was provided by TWC participants (see document TWC/37/12 “Report”, paragraphs 73 and 92).

Summary of crop and authorities currently using biochemical and molecular techniques

Argentina	Soybean
Brazil	*Eucalyptus*, Soybean
China	Broccoli, Cauliflower, Chinese cabbage, Eggplant, Lettuce, Maize, Pepper, Rice, Rose, Sorghum, Strawberry, Walnut, Wheat, Fruit trees, Ornamentals, Soybean, Cotton, and other 29 crops
Denmark	Barley, Oats, Rye, Wheat, Forage grasses
European Union	Lettuce, Maize, Potato, Wheat, Vegetable, Barley, Sunflower
France	Maize, Oilseed rape
Italy	Soybean, Rice
Japan	Rice, Green tea, Strawberry, Japanese pear, French bean, Sweet cherry, Apple, Lettuce
Netherlands	French bean, *Phalaenopsis*, Potato, Rose, Tomato
Republic of Korea	Chinese cabbage, Cucumber, Lettuce, Melon, Pepper, Pumpkin, Radish, Rice, Tomato
Russian Federation	Maize, Potato, Soybean, Sunflower, Wheat
United Kingdom	Barley, Potato, Oilseed rape

Summary of current use of biochemical and molecular techniques

Use:
Management of variety collection and selection of similar varieties
Validation of male sterility and disease resistance
Validation of DUS/VCU samples
Variety identification
Research purposes
Breeding
Techniques:
ALFP (NL)
CAPS (JP)
MNP (CN)
OSR-SSR (FR)
PRG-SNPs (NL)
RAPID – STS (JP)
SSR (BR, CN, DK, GB, IT, JP, KR, NL, QZ)
SNPs (AR, CN, FR, DK, GB, NL, QZ)

Summary of databases with molecular marker information, by crops

Argentina	Soybean (under development)
China	Apple, Cotton, Maize (for research), Pepper, Rice, Rose, Sorghum, Soybean, Walnuts, Wheat, Fruit trees
Denmark	Barley, Wheat, Forage grasses
European Union	Potato
France	Maize
Italy	Soybean
Netherlands	French bean, *Phalaenopsis*, Potato
United Kingdom	For research

Developments at the eighteenth session of the Working Group on Biochemical and Molecular Techniques, and DNA-Profiling in Particular

15. The BMT, at its eighteenth session, considered document BMT/18/5 “Session to facilitate cooperation” and formed discussion groups to allow participants to exchange information on their work on biochemical and molecular techniques and explore areas for cooperation. The following information was provided by the participants (see document BMT/18/21 “Report”, paragraphs 38 and 41).

Maize and Soybean

Summary of crop interest

Maize	China, Germany, Kenya, Russian Federation, ISTA, SAA
Soybean	Argentina, Brazil, China, ISTA

Plans for cooperation

- Argentina will publish a set of 4004 SNP markers for the management of variety collections in Soybean and will inform Brazil and the United States of America with a view to their testing the discriminating power of this set.
- Brazil to discuss with the Brazilian breeders association the proposal on the use of molecular markers in DUS examination for soybeans (e.g. similar to the study conducted in Argentina).
- China to make the new Maize 6H-60K SNP chip available for testing .

Summary of current use of biochemical and molecular techniques

Germany: isoenzymes for management of variety collection and DUS examination (maize)
China: Maize 6H-60K SNP chip for consideration of essential derivation; protocol for variety identification in maize and soybean; creation of a database and selection of similar varieties; general protocol for variety identification using SSR
Argentina: SNP for management of variety collection and variety identity
Brazil: SSR for variety identity
SAA: genetic similarity in soybean varieties
ISTA: electrophoresis, seed proteins, SSR (ISTA Rules, Chapter 8)

Proposals on confidentiality and access to data

- DNA-fingerprint data to be treated as confidential;
- Variety identification data using a small number of SNP markers could be made publicly available
- Consent by the breeder should be required before sharing of DNA-based information;
- Breeders should be informed about the publication of variety identification by SNPs;
- Parental line information should be treated as confidential

Other agricultural crops

Summary of crop interest

Barley	Argentina, Estonia, Germany, Italy, United Kingdom, ISTA
Cannabis sativa	Estonia, Italy, Netherlands, United Kingdom
Cotton	Argentina, ISTA
Perennial Ryegrass	Germany, Netherlands, New Zealand, United Kingdom
Potato	Estonia, Germany, Netherlands, Russian Federation, United Kingdom
Rice	Argentina, China, Italy, Japan, ISTA
Sunflower	Russian Federation
Sweet Potato	United Kingdom
Wheat	Argentina, China, Estonia, Germany, Italy, United Kingdom, ISTA

Plans for cooperation

- Ryegrass: Belgium, Czech Republic and the Netherlands to share information on their work and plans;
- Oilseed rape: France, Germany, CPVO and the United Kingdom to develop a set of molecular markers for the management of variety collections;
- INVITE and INNOVAR (scope of 10 crops) participating countries to develop markers sets for variety testing;
- Argentina to contact BMT participants on sets of markers for Barley, Cotton, Rice and Wheat.

Summary of current use of biochemical and molecular techniques

Netherlands and the United Kingdom: SNPs for management of variety collections
China: 90K SNP chip for wheat; development of testing standard for SSR in wheat; creation of a database for wheat varieties; SSR markers for selection of similar varieties and variety purity
Germany: electrophoresis for Barley, Wheat and Oat, Ryegrass, Potato for DUS examination
Italy: electrophoresis in maize, sunflower, wheat, barley for DUS examination and variety identification; SSR for variety hybridity in Rice and variety identification
Japan: RAPD-STS markers for infringement cases in French Bean and Rice
Russian Federation: SSR for identification in Sunflower and Potato.
United Kingdom: electrophoresis for Barley, Wheat and Oat, Ryegrass, for DUS examination; SSR and SNP for sample validation and variety identification
ISTA: maize, wheat and soybean: SSR and electrophoresis; barley: SSR; Other crops: electrophoresis

Proposals for confidentiality and access to data

Participants at the discussion group on other agricultural crops agreed with the proposals by the discussion group on Maize and Soybean.

Vegetables

Summary of crop interest

Cabbage	China, Republic of Korea
Chinese cabbage	China, Republic of Korea
Cucumber	China, Netherlands, Republic of Korea
Eggplant	Italy
French bean	Netherlands
Lettuce	Australia, Italy, Netherlands, Republic of Korea
Melon	China, Netherlands, Republic of Korea
Onion	Italy, Netherlands
Oriental melon	Republic of Korea
Pea	Netherlands, United Kingdom
Pepper	China, Italy, Netherlands, Republic of Korea
Pumpkin	Republic of Korea
Radish	Republic of Korea
Shallot	Netherlands
Squash	Italy
Tomato	China, France, Italy, Japan, Netherlands, Republic of Korea
Watermelon	China, Italy, Republic of Korea

Summary of current use of biochemical and molecular techniques

Use:
Research (NL)
TGP/15 Model 1 (JP, NL, FR)
French bean example (NL)
Variety identifications (CN, IT, NL)
Techniques:
AFLP (NL)
Capillary electrophoresis fragment analysis (IT)
MNP (CN)
SNPs (NL, CN, IT)
SSR (CN, IT)
Taqman (NL)
Whole genome sequencing / GBS (CN, NL)

Proposals for confidentiality and access to data

The discussion group on vegetables agreed to propose inviting breeders, observer organizations and other participants to make presentations on ownership matters during the breeders' day at the nineteenth session of the BMT.

Ornamental plants

Summary of crop interest

Bougainvillea	China
Camellia	China
Chrysanthemum	China, Netherlands
Gypsophila	Netherlands
Helleborus	Netherlands
Hibiscus	China
Hydrangea	France
Lilium	China
Phalaenopsis	Netherlands
Rose	China, Netherlands, CIOPORA
Tree Peony	China

Plans for cooperation

- Rose: China, Netherlands and CIOPORA to discuss a methodology for validating a set of molecular markers between laboratories.
- Chrysanthemum, Rose, Tree peony: China to explore cooperation on developing molecular markers with other UPOV members.

Summary of current use of biochemical and molecular techniques

Use:
Variety identification (CN)
Research (CN, FR)
Techniques:
SSR (CN, FR)
SNPs (CN)

Proposals on confidentiality and access to data

- To develop an agreement template with breeders for the use of molecular data. The template should include a requirement for a description of the intended use of the data.

Fruit crops and forest trees

Summary of crops of interest

Citrus	China, Italy, Spain
Persimmon	Spain, Republic of Korea
Peach	Italy, Hungary, Spain
Strawberry	Italy, Hungary, Spain
Goji Berry	China
Walnut	China

Plans for cooperation

Citrus – under consideration	Spain to propose collaboration initiative with Italy
Persimmon	Spain, Republic of Korea
Peach	Italy, Hungary
Strawberry – under consideration	Italy, Hungary

Summary of current use of biochemical and molecular techniques

Australia: possible use of microsatellites in some enforcement cases.
China: SSR markers for variety identification in Apple, Chinese Dates, Citrus, Apricot, Goji Berry and Fraxinus
European Union: collaboration on epigenetic markers in apple;
Japan: considering the use of SSR for enforcement for grapes and CAPS for citrus.
Republic of Korea: SSR for Apple, Peach, Grape, Pear and persimmon.
Spain: SSR for variety identification; use of SNP for research, including DUS testing

Proposals on confidentiality and access to data

New Zealand has published position on access and use of plant material including molecular data. For example, molecular data would only be provided with permission of breeder.

REVIEW OF DOCUMENT UPOV/INF/17 "GUIDELINES FOR DNA-PROFILING: MOLECULAR MARKER SELECTION AND DATABASE CONSTRUCTION ('BMT GUIDELINES')

16. The BMT, at its eighteenth session, considered documents BMT/18/10 "Review of document UPOV/INF/17 "Guidelines for DNA-Profiling: Molecular Marker Selection and Database Construction ('BMT Guidelines')"" and UPOV/INF/17/2 Draft 2 "Guidelines for DNA-Profiling: Molecular marker selection and database construction ('BMT Guidelines')" as a basis for the revision of document UPOV/INF/17, and agreed the following changes to UPOV/INF/17/1 (see document BMT/18/21 "Report", paragraphs 43 to 68).

Section A. Introduction

17. The BMT agreed to amend the text of the Introduction to read as follows:

"The purpose of this document (BMT Guidelines) is to provide guidance ~~for developing~~ on harmonized ~~methodologies~~ principles for the use of molecular markers with the aim of generating high quality molecular data for a range of applications. Only DNA molecular markers are considered in this document.

"The BMT Guidelines are also intended to address the construction of databases containing molecular profiles of plant varieties, possibly produced in different laboratories using different technologies. In addition, the aim is to set high demands on the quality of ~~the~~ markers and on the desire for generating reproducible data using these markers in situations where equipment and/or reaction chemicals might change. Specific precautions need to be taken to ensure quality entry into a database. "

Section B. General Principles

18. The BMT agreed to add the following text to the Section B:

"For DNA profiling of a plant variety, a set of molecular markers and a method to detect them are required. Two different sets of molecular markers detected with the same method will result in two different DNA profiles for a particular variety. In contrast, two different methods to detect the specific alleles of a given molecular marker set are expected to result in identical DNA profiles. Standardization of the detection method and technology is not required as long as the performance meets the quality criteria and the resulting DNA profiles are consistent. Irrespective of the technology used to detect defined marker sets, the genotype of a particular variety should not be affected.

"Molecular marker sets, marker detection methods and subsequently the database developmental process can be subdivided into 5 different phases:

1. Selection of molecular markers
2. Selection of detection method
3. Validation and harmonization of the detection method
4. Construction of the database
5. Data exchange

"This document describes these different phases in more detail. It is considered that these phases are independent on the stage of development of genotyping technologies and future improvements in high-throughput sequencing. "

19. The BMT agreed that phase 5: "data exchange" should be clarified in the proposed text.

Section 1. Selection of a Molecular Marker Methodology

20. The BMT agreed to delete current Section 1 from Document UPOV/INF/17/1.

New Section 1.1 Sets of varieties for the selection process

21. The BMT agreed to add new Section 1.1 "Sets of varieties for the selection process" with the following text:

"For DNA profiling of plant varieties and database construction, molecular markers should be selected according to the objective. To start the marker selection process an appropriate number of varieties (development set) is needed to reflect the diversity observed within the group/crop/species/type for which the markers are intended to be discriminative. Further selection is performed by profiling additional varieties (validation set) to measure the performance of the markers. Criteria for the choice of the validation set could be:

(a) genetically very similar varieties or lines, NILs, RILs
(b) parental lines and offspring
(c) genetically close but morphologically distinct varieties (e.g. mutants)
(d) some morphologically close varieties with different pedigree
(e) different lots of the same variety
(f) different origins of the same variety"

New Section 1.2 Molecular markers – performance considerations

22. The BMT agreed to amend the new Section 1.2 to read as follows:

"The following general criteria for ~~choosing~~ selecting a specific marker or set of markers are intended to be appropriate ~~for molecular markers~~ irrespective of the use of the markers, although it is recognized that specific uses may impose certain additional ~~criteria~~ considerations:

(a) ~~useful level of polymorphism;~~ Number of markers should be balanced with the accuracy of the genotype required for the objective. The number of markers to reach the necessary resolution or discriminative power depends on marker-type (dominant/co-dominant; bi-/multi-allelic), species and the quality of the marker performance;

(b) repeatability, reproducibility and robustness within and between, laboratories in terms of scoring data;

(c) ~~known distribution of the markers throughout the genome (i.e. map position), which whilst not being essential, is useful information and helps to avoid the selection of markers that may be linked~~ Coverage of the genome and the linkage disequilibrium should reflect the objectives. Knowing the physical and/or genetic position of the selected markers on the genome is not essential but enables a good selection of markers; ~~and~~

(d) Possible sources of molecular markers
- Molecular markers derived from public resources
- Molecular markers derived from non-public resources, screening and selection of commercially available species-specific chips and arrays.
- Molecular markers selected from newly generated sequence data;

(e) the avoidance, as far as possible, of markers with "null" alleles (i.e. an allele whose effect is an absence of a PCR product at the molecular level), which again is not essential, but advisable~~.~~;

(f) Allowance of easy, objective and indisputable scoring of marker profiles. These good performing markers are preferred over complex marker profiles that are sensitive to interpretation. Clear black and white answers also allows for easier harmonization;

(g) Co-dominant markers are generally preferred over dominant markers as they have a higher discriminative power;

(h) Durability of the marker. When a marker is located in a genomic area that is not subject to selection by breeders, there is a better chance that the marker will be informative in a durable way;

(i) Markers could be located in coding and/or in non-coding regions; and

(j) The use of molecular markers is species-specific and should take into account the features of propagation of the species."

Section 2.2 Criteria for specific types of molecular markers

23. The BMT agreed to delete current Section 2.2 from Document UPOV/INF/17/1

New Section 2.1 DNA profiling methods - general considerations

24. The BMT agreed to add the new Section 2.1 under the new Section 2 "Selection of the Detection Method" with the following text:

"2.1 DNA profiling methods - general considerations

"2.1.1 Important considerations for choosing DNA profiling methods that generate high quality molecular data are:

(a) reproducibility of data production within and between laboratories and detection platforms (different types of equipment);
(b) repeatability over time;
(c) discrimination power of the method;
(d) time and labour intensity of the method;
(e) robustness of performance in time and conditions (sensitiveness to subtle changes in the protocol or condition);
(f) flexibility of the method, possibility to vary in the number of samples and/or number of markers;
(g) interpretation of the data produced is independent of the equipment;
(h) sustainability of databases;
(i) accessibility of methodology;
(j) independent of a specific machine, specific chemistry, specific supplier, particular partners or products;
(k) suitable for automation;
(l) suitable for multiplexing; and
(m) cost effective; costs, number of samples and number of markers are in balance."

New Section 3. Validation and harmonization of a marker set and detection method

25. The BMT agreed to add the new Section 3 with the following text:

"3.1 Validation and harmonization – general considerations

Molecular marker selection and detection method descriptions are based on performance: markers and methods should be robust and give rise to consistent DNA profiles. Performance of molecular markers and genotyping methods is evaluated in a validation process. In case of shared databases, consistency of the DNA profiles in different laboratories is evaluated in the harmonization process using different equipment and chemistries. The usage of validated markers and methods will lead to harmonized results.

"3.2 Performance considerations - validation of markers and methods

It is needed to determine how suitable the selected marker set is (fit-for-purpose). The accuracy should be measured. To determine the adequacy of a method and DNA marker set several points should be considered:

(a) Discriminative capacity/informativeness;
(b) Repeatability;
(c) Reproducibility;
(d) Robustness; and
(e) Error-rate.

"3.3 Consistency considerations - harmonization of markers and methods between different laboratories in case of a shared database – ring test

(a) Use defined collection of varieties representing a wide range of alleles as a reference in all labs to test consistency between labs

(b) Duplicates, sub-samples, individual plants of a variety to check the consistency of the DNA profiles and estimate the error-rate between labs

(c) Agreements on the scoring of molecular data. The necessity to develop a protocol for allele/band scoring between labs depends on the used marker type (e.g. essential for SSR but less urgent for SNP markers). The protocol could address how to score the following:

i. rare alleles (i.e. those at a specific locus which appear with a frequency below an agreed threshold (commonly 5-10%) in a population);

ii. null alleles (an allele whose effect is an absence of PCR product at the molecular level);

iii. "faint" bands (i.e. bands where the intensity falls below an agreed threshold of detection, set either empirically or automatically, and the scoring of which may be open to question);

iv. missing data (i.e. any locus for which there are no data recorded for whatever reason in a variety or varieties); and

v. monomorphic bands or non-informative allele scores (those alleles/bands which appear in every variety analysed, i.e. are not polymorphic in a particular variety collection)."

26. The BMT agreed that the European Union, France and the Netherlands should prepare definitions of the terminology in the new Section 3.2 as footnotes.

Section 5. Standardization of Analytical Protocols

27. The BMT agreed to delete Section 5.

New Section 4. Construction of a Crop-specific Database

28. The BMT agreed to add the new Section 4 with the following text:

"The data that is stored in a database and how it is stored should reflect the process of producing the data. Therefore, database construction should consider different levels of data processing (ie. raw data, sequence data…). The database should store: 1) the end results, e.g. the DNA profile as well as how it was derived both in terms of; 2) laboratory method description and 3) the computational steps for deriving a DNA profile."

New Section 4.1

29. The BMT agreed to add the new Section 4.1 with the following text:

"4.1 Recommendations for database design

Design of databases could consider the following aspects:

(a) The database architecture should be flexible, e.g. allow for storing both flat files as well as compressed archives.

(b) Contain different tables. Separate tables and entries are required for laboratory experimental work, data processing and the allele scores.

(c) Store information at different levels (allele scores / how the allele score was called (the rules or the interpretation rules behind a decision) / (links) to the raw data (tiff files, bam files, files that came out of the machine that produced the data that were used for allele scoring and interpretation).

(d) For sequencing data, variant call files in VCF or BCF format corresponding to the standard version 4.2 or higher. Header entries should contain the name and version of the different scripts used for both sequence read mapping, read filtering, variant calling and variant filtering in such a way that a bioinformatician can repeat the analysis.

(e) In case of replicate samples, one genotype entry can be computed and stored in case the DNA profiles of the replicates match. In case of non-matching replicates, the record needs to be flagged or filtered out where appropriate. The rules applied for these cases need to be documented in a publicly accessible code repository that is references from the variant call file. Frequencies could also be used for heterogeneous varieties.

(f) Validation of the VCF and or BCF data against relevant specifications.

(g) Easy to share data, (e.g. API). "

New Section 4.2

30. The BMT agreed to amend the new Section 4.2 "Requirements of the plant material" to read as follows:

"*4.2 Requirements of the plant material*

"The source and type of the material and how many samples ~~need~~ to be ~~analyzed~~ stored and shared in the database are the main issues with regard to the material to be analyzed.

"4.2.1 Source of plant material

"The plant material to be analyzed should be an authentic, representative sample of the variety and, ~~where~~ when possible, should be obtained from the sample of the variety used for examination for the purposes of Plant Breeders' Rights or for official registration. Use of samples of material submitted for examination for the purposes of Plant Breeders' Rights or for official registration will require the permission of the relevant authority, breeder and/or

maintainer, as appropriate. The plant material from which the samples are taken should be traceable in case some of the samples subsequently prove not to be representative of the variety.

"4.2.2 Type of plant material

"The type of plant material to be sampled and the procedure for sampling the material for DNA extraction will, to a large extent, depend on the crop or plant species concerned. For example, in seed-propagated varieties, seed may be used as the source of DNA, whereas, in vegetatively propagated varieties, the DNA may be extracted from leaf material. Whatever the source of material, the method for sampling and DNA extraction should be ~~standardized and~~ documented. Furthermore, it should be verified that the sampling and extraction methods produce consistent results by DNA analysis.

"4.2.3 Sample size and type (bulk or individual samples)

"It is essential that the samples taken for analysis are representative of the variety and well documented. With regard to being representative of the variety, consideration should be given to the features of propagation (see the General Introduction). ~~The size of the sample should be determined taking into account suitable statistical procedures.~~

"4.2.4 DNA reference sample

"~~It is recommended that~~ A DNA reference sample collection ~~should~~ may be created from the plant material sampled ~~according to sections 4.1 to 4.3. This has the benefit that the DNA reference samples can be stored and supplied to other laboratories.~~ The method for sampling should follow recommended procedures and DNA extraction should fit some quality criteria. Both need to be documented.

"The DNA samples should be stored in such a way as to prevent degradation (e.g. storing it at -80C). The transfer of DNA reference samples is described in document TGP/5: section 1."

New Section 4.3 Processing of sequence data

31. The BMT agreed to add the new Section 4.3 "Processing of sequence data" with the following text:

*"*A detailed log of the data processing pipeline may include:

(a) type and versions of tools;
(b) command line used for the tool including thresholds;
(c) reproducibility counts:
(d) possibility for sharing the data and process;
(e) raw alignment data (BAM or CRAM files) should be stored where possible;
(f) one VCF file per variety must be present, multi-sample VCF files are not suitable;
(g) if VCF files are stored, all positions (both variants & non-variants) and their depth should be stored;
(h) both heuristic and probabilistic approaches should be considered and compared for detection methods;
(i) databases should facilitate input and output of variant call data in standardized format (VCF or BCF);
(j) the data processing pipeline should result in a detailed log file which should be stored in conjunction to the variant call data;
(k) if possible, raw data should be stored so that data processing can be repeated with new or updated tools; and
(l) a p-value or uncertainty for a given allele should be stored."

New Section 4.4 Type of database

32. The BMT agreed to amend the new Section 4.4 "Type of database" to read as follows:

"There are many ways in which molecular data can be stored, therefore, it is important that the database structure is developed to be compatible with all intended uses of the data. For molecular data obtained using next generation sequencing (NGS), the variant call file standard VCFv4.2 can be used. "

New Section 4.5 Database model

33. The BMT agreed to amend the text of the new Section 4.5 "Database model" to read as follows:

"The database model should be defined by IT database experts in conjunction with the users of the database. As a minimum the database model should contain six core objects: Species; Variety; ~~Technique~~ Marker detection method **Error! Bookmark not defined.**; Marker; Locus; and Allele. For variants obtained from sequencing data, VCF files can be stored in a relational or non-SQL database. In this case, each database record for a variant has a defined genome version, chromosome, position, reference allele. "

New Section 4.6.1

34. The BMT agreed to amend the new Section 4.6.1 to read as follows:

"4.6.1 In a database, each of the objects becomes a table in which fields are defined. For example:

(a) ~~Technique/Marker code~~ Marker type: indicates the code or name of the technique or type of marker used, e.g. SSR, SNP, etc.

(b) Reference genome position / Locus code: Preferably, a genome assembly version, chromosome and position should be provided if a reference genome is available for the species concerned, e.g. SL2.50ch05:63309763 for tomato *Solanum lycopersicum* assembly version 2.50 on chromosome 5 position 63309763. If no reference genome is available or the location is unknown, a ~~indicates~~ name or code of the locus for the species concerned can be used, e.g. gwm 149, A2, etc.

(c) ~~Allele code~~ Genotype: For SNP profiles, the allele composition of the SNP or MNP should be given, e.g. A/T or A/A. For other techniques, genotype indicates the name or code of the allele of a given locus for the species concerned, e.g. 1, 123, etc.

(d) Allele depths / Data value: For SNPs obtained from next generation sequencing data, the depth of coverage for alleles should be indicated (e.g. 10/20 for an A/T allele in which the A is covered by 10 reads and the T by 20). Otherwise, a data value for a given sample on a given locus-allele should be indicated, e.g. 0 (absence), 1 (presence), 0.25 (frequency) etc.

(e) Variety: Variety denomination or breeder's reference: the variety is the object for which the data has been obtained. ~~Grouping~~Type of variety: e.g. Inbred Line or Hybrid

(f) Species: the species is indicated by the botanical name or the national common name, which sometimes also refers to the type of variety (e.g. use, winter/spring type etc.). The use of the UPOV code would avoid problems of synonyms and would, therefore, be beneficial for coordination."

Section 6.

35. The BMT agreed to delete Sections 6.4, 6.5, 6.6, 6.7 and 6.8.

36. The BMT agreed that the text in the Section 6.6 "Data access / ownership" should be reinstated.

New Section 5. Data Exchange

37. The BMT agreed that general sentences of the new Section 5 should be kept in the main document, while the text of technical details in this Section should be put in the Annex to a new draft.

38. The BMT agreed that data transfer methods should be mentioned in a new draft. China is invited to provide a draft on data transfer methods with examples to the European Union, France and the Netherlands.

Summary

39. The BMT agreed to amend the Summary to read as follows:

"A detailed log of the data processing pipeline may include:

"The following is a summary of the approach recommended for high quality DNA profiling of varieties including the selection and use of molecular markers ~~to construct central~~ as well as the construction of shared and sustainable molecular databases ~~of DNA profiles of varieties~~ (i.e. databases that can be populated in the future with data from a range of sources, independent of the technology used).

(a) consider the approach on a crop-by-crop basis;
(b) agree on an acceptable marker type and source;
(c) agree on acceptable detection platforms/equipment;
(d) agree on laboratories to be included in the test;
(e) agree on quality issues ~~(see section 5.2)~~;
(f) verify the source of the plant material used ~~(see section 4)~~;

(g) agree which markers are to be used in a preliminary collaborative evaluation phase, involving more than one laboratory and different detection equipment ~~(see section 2)~~;
(h) conduct an evaluation ~~(see section 5.3)~~;
(i) develop a protocol for scoring the molecular data ~~(see section 5.4)~~;
(j) agree on the plant material/reference set to be analyzed, and the source(s);
(k) analyze the agreed variety collection, in different laboratories/different detection equipment, using duplicate samples, and exchanging samples/DNA extracts if problems occur;
(l) use reference varieties/DNA sample/alleles in all analyses;
(m) verify all stages (including data entry) – automate as much as possible;
(n) conduct a 'blind test' in different laboratories using the database;
(o) adopt the procedures for adding new data."

GLOSSARY

40. The BMT agreed to delete the Glossary.

New Section C LIST OF ACRONYMS

41. The BMT agreed to add the list of acronyms with the following text:

"BAM	Binary Alignment Map
BCF	Binary Call Format
CRAM	Compressed Reference-oriented Alignment Map
MNP	Multiple Nucleotide Polymorphism
NIL	Near Isogenic Line
RIL	Recombinant Inbred Line
SAM	Sequence Alignment Map
SNP	Single Nucleotide Polymorphism
TIFF	Tagged Image File Format
VCF	Variant Call Format"

42. The BMT agreed to propose to the TC that the European Union, France and Netherlands prepare a new draft of INF/17 for consideration of the nineteenth session of the BMT (see document BMT/18/21 "Report", paragraph 69).

[End of document]