UPOV

International Union for the Protection of New Varieties of Plants

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Working Group on Biochemical and Molecular Techniques and DNA-Profiling in Particular

BMT/18/21

Eighteenth Session
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REPORT

Adopted by the Working Group on Biochemical and Molecular Techniques and DNA-Profiling in Particular

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Opening of the session

1. The Working Group on Biochemical and Molecular Techniques and DNA-Profiling in Particular (BMT) held its eighteenth session in Hangzhou, China, from October 16 to 18, 2019. The list of participants is reproduced in Annex I to this report.

2. The session was opened by Mr. Nik Hulse (Australia), Chairperson of the BMT, who welcomed the participants and thanked China for hosting the BMT session.

3. The BMT was welcomed by Mr. Jianmeng Li, Division Director, Seed and Innovation Division, Ministry of Agriculture and Rural Affairs (MARA), China.

4. The BMT received a presentation by Mr. Ruixi Han, Senior Examiner, Division of DUS Tests, Development Center of Science and Technology, Ministry of Agriculture and Rural Affairs, China, on "Using Molecular Techniques in DUS tests and PVP enforcement in China". A copy of the presentation is provided in Annex II to this report.

Adoption of the agenda

5. The BMT adopted the agenda as reproduced in document BMT/18/1 Rev., with a change of order to the following: 1, 2, 3, 4, 7, 10, 12, 5, 14, 6, 15, 8, 9, 11, 13, 16, 17, 18, 19.

Reports on developments in UPOV concerning biochemical and molecular techniques

6. The BMT received a presentation from the Office of the Union on developments in UPOV concerning biochemical and molecular techniques, a copy of which is provided in document BMT/18/2.

Short presentations on new developments in biochemical and molecular techniques by DUS experts, biochemical and molecular specialists, plant breeders and relevant international organizations

7. No documents were received for this agenda item.

Variety description databases including databases containing molecular data

Advances in the construction and application of DNA fingerprint database in maize

8. The BMT received a presentation "Use of SSR and SNP markers in maize variety identification" by Ms. Rui Wang (China), a copy of which would be provided as document BMT/18/6 Rev..

Report on development of a software tool for marker selection using the traveling salesman algorithm

9. The BMT considered document BMT/18/11 and received a presentation by Mr. Barry Nelson (Seed Association of the Americas (SAA)), a copy of which would be provided as an addendum to BMT/18/11.

10. The BMT agreed to invite members to test the software for the selection of panels of molecular markers for variety identification and report to the BMT, at its nineteenth session.

The use of molecular techniques in variety identification

(a) Applications of MNP marker in plant varieties protection

11. The BMT received a presentation by Mr. Hai Peng (China), a copy of which is reproduced in document BMT/18/15.

(b) Association Analysis of SSR Markers and Agronomic Traits in Soybean

12. The BMT received a presentation by Ms. Dongmei Li (China), a copy of which would be provided in BMT/18/19 Rev..

Report of work on molecular techniques in relation to DUS examination

Facilitating Distinctness, Uniformity and Stability Testing of Soybean Varieties: Development and Validation of Molecular Marker and Variety Sampling Methodologies

13. The BMT considered document BMT/18/8 and received a presentation by Mr. Frédéric Achard (SAA), a copy of which would be provided as an addendum to document BMT/18/8.

Facilitating Distinctness, Uniformity and Stability Testing of Soybean Varieties: Establishing Criteria for the use of Single Nucleotide Polymorphism data

14. The BMT considered document BMT/18/9 and received a presentation by Mr. Paul T. Nelson (SAA), a copy of which would be provided as an addendum to document BMT/18/9.

Next generation variety testing for improved cropping on European farmland (InnoVar)

15. The BMT considered document BMT/18/12 and received a presentation by electronic means from Ms. Lisa Black (United Kingdom), a copy of which would be provided as an addendum to document BMT/18/12.

CPVO report on IMODDUS: latest developments (INVITE) and update on R&D projects

16. The BMT received a presentation by Ms. Cécile Collonnier (European Union), a copy of which would be provided as document BMT/18/14 Rev..

A simple SSR based identification system for sweet potato

17. The BMT considered document BMT/18/16 and received a presentation by Mr. Alex Reid (United Kingdom), a copy of which would be provided as an addendum to document BMT/18/16.

Use of molecular markers for protection and varietal identification: state of the art in Argentina

18. The BMT considered document BMT/18/17 and received a presentation by Mr. Mariano Mangieri (Argentina), a copy of which would be provided as an addendum to document BMT/18/17.

What information is essential for "character-specific molecular markers" in Test Guidelines

19. The BMT received a presentation by Ms. Hedwich Teunissen (Netherlands), a copy of which is reproduced in document BMT/18/18.

Revision of document TGP/15 "Guidance on the Use of Biochemical and Molecular Markers in the Examination of Distinctness, Uniformity and Stability (DUS)"

20. The BMT considered document BMT/18/7.

Characteristic-specific marker with incomplete information on state of expression

21. The BMT agreed with the proposed example to be added to document TGP/15 to illustrate a situation where the characteristic-specific marker does not provide complete information on the state of expression of a characteristic, as set out in document BMT/18/7, the Annex II, with the following amendments:

(a) to amend paragraph 2 of the proposed example to read as follows:

"2. Resistance to ToMV Strain 0 is conferred by the presence of ~~one or more genes, including~~ alleles *Tm1*~~*,*~~ *Tm2*~~*;*~~ and *Tm2*^{2} from genes Tm1 and Tm2."

(b) to add the following text at the end of paragraph 6 of the proposed example:

"If a variety is claimed to be susceptible to ToMV Strain 0, a bioassay should be performed to confirm the claim."

Cooperation between international organizations

22. The BMT considered document BMT/18/4.

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

23. The BMT agreed that relevant elements from the World Seed Partnership and the FAQ on the use of molecular techniques in the examination of DUS, as presented in Annexes I and II of document BMT/18/4, 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.

Inventory on the use of molecular marker techniques, by crop

24. The BMT considered the elements for an inventory on the use of molecular marker techniques, by crop, as set out in document BMT/18/4, paragraph 25.

25. 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.

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

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

28. 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).

29. 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").

30. 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.

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

32. The BMT noted that, on the basis of the comments received from the TWPs and BMT, proposed elements for the inventory on the use of molecular marker techniques would be presented for consideration by the TC at its fifty-fifth session.

33. The BMT noted that, subject to agreement by the TC, at its fifty-fifth session, and in coordination with the OECD, a circular would be issued to request members of the Union to complete a survey as a basis to develop an inventory on the use of molecular marker techniques.

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

34. In response to the request to develop lists of possible joint initiatives with OECD and ISTA, in relation to molecular techniques, the BMT 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.

(a) Horizontal methods for molecular biomarker analysis

35. The BMT received a presentation by electronic means from Mr. Michael Sussman (International Organization for Standardization (ISO)), a copy of which is reproduced in document BMT/18/13.

(b) OECD Seed Scheme: an international seed varietal certification system

36. The BMT received a presentation by Ms. Kristiina Digryte (Organisation for Economic Co-operation and Development (OECD)), a copy of which is reproduced in document BMT/18/20.

(c) International Seed Testing Association

37. The BMT received a presentation by Mr. Keshavulu Kunusoth (International Seed Testing Association (ISTA)), a copy of which is reproduced in document BMT/18/21.

Session to facilitate cooperation

38. The BMT considered document BMT/18/5.

39. The BMT considered how the outcomes of the discussion held at the TWPs at their sessions in 2019, on cooperation in relation to use of molecular techniques, might feed into the work of the BMT.

40. The BMT received a presentation on “Access to reference material and data from EU examination offices” from an expert from European Union, a copy of which would be provided in an addendum to document BMT/18/5.

41. The BMT 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.

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

42. 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

43. 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

44. 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.

Management of databases and exchange of data and material; Methods for analysis of molecular data; The use of molecular techniques in examining essential derivation

45. No documents were received for these agenda items.

Review of document UPOV/INF/17 "Guidelines for DNA-Profiling: Molecular Marker Selection and Database Construction"

46. The BMT considered documents BMT/18/10 and UPOV/INF/17/2 Draft 2 as a basis for the revision of document UPOV/INF/17, and agreed the following changes to UPOV/INF/17/1.

Section A. Introduction

47. 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

48. 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."

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

Section 1. Selection of a Molecular Marker Methodology

50. 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

51. 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

52. 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

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

New Section 2.1 DNA profiling methods - general considerations

54. 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

55. 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)."

56. 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

57. The BMT agreed to delete Section 5.

New Section 4. Construction of a Crop-specific Database

58. 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

59. 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

60. 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

61. 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

62. 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

63. 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

64. 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.

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

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

New Section 5. Data Exchange

67. 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.

68. 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

69. 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

70. The BMT agreed to delete the Glossary.

New Section C LIST OF ACRONYMS

71. 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"

72. 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.

Date and place of next session

73. At the invitation of the United States of America, the BMT agreed to hold its nineteenth session in Alexandria, Virginia, jointly with TWC, during the week of September 21, 2020.

Future program

74. During its nineteenth session, the BMT planned to discuss the following items:

1. Opening of the session

2. Adoption of the agenda

3. Reports on developments in UPOV concerning biochemical and molecular techniques (document to be prepared by the Office of the Union)

4. Short presentations on new developments in biochemical and molecular techniques by DUS experts, biochemical and molecular specialists, plant breeders and relevant international organizations (oral reports by participants)

5. Report of work on molecular techniques in relation to DUS examination (papers invited)

6. Cooperation between international organizations (document to be prepared by the Office of the Union)

7. Variety description databases including databases containing molecular data (papers invited)

8. Methods for analysis of molecular data, management of databases and exchange of data and material (papers invited)

9. The use of molecular techniques in examining essential derivation[1] (papers invited)

10. The use of molecular techniques in variety identification[1] (papers invited)

11. Confidentiality, ownership and access to molecular data[1] (papers invited)

12. Review of document UPOV/INF/17 “Guidelines for DNA-Profiling: Molecular Marker Selection and Database Construction

13. Session to facilitate cooperation

14. Date and place of next session

15. Future program

16. Report of the session (if time permits)

17. Closing of the session

75. The BMT considered the organization of the TWC and BMT meetings on the same week. The BMT agreed with the TWC there was a duplication of content presented at the TWC and BMT meetings and agreed there should be a single opening and introductory parts for both meetings at the same time.

76. The BMT agreed with the TWC that the above proposals could enable the allocation of time during the meeting for a technical visit.

77. The BMT adopted this report at the end of the session.

[Annexes follows]

[1] Breeders’ Day

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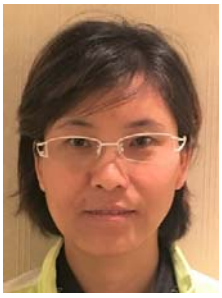

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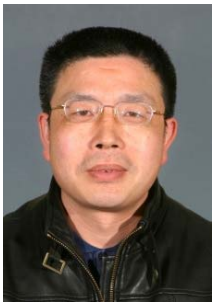

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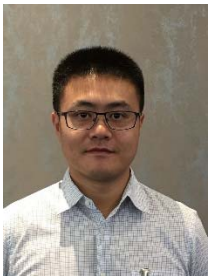

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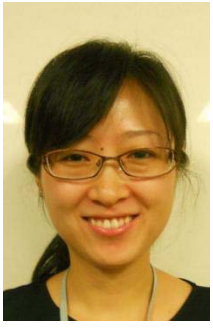

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[Annex II follows]

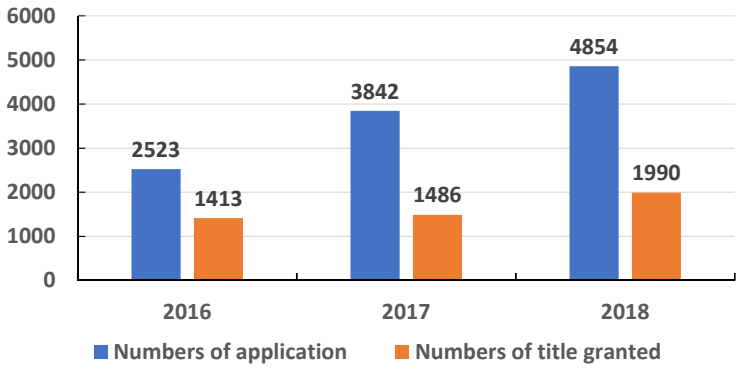
Overview of using Molecular Techniques in DUS test and PVP enforcement in China

HAN Ruixi

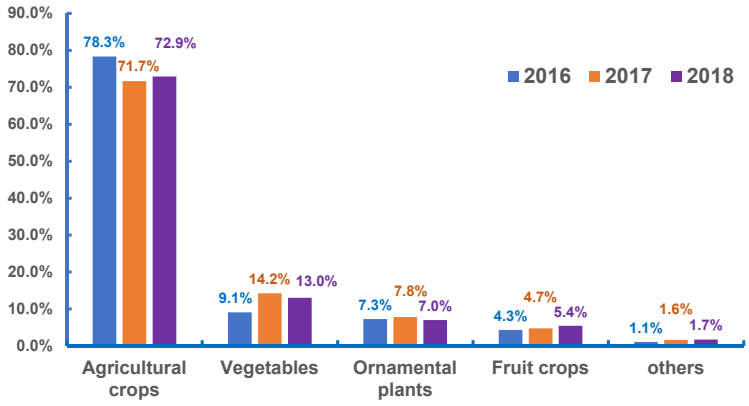
October 16, 2019 Hangzhou

Development Center of Science and Technology,
Ministry of Agriculture and Rural Affairs

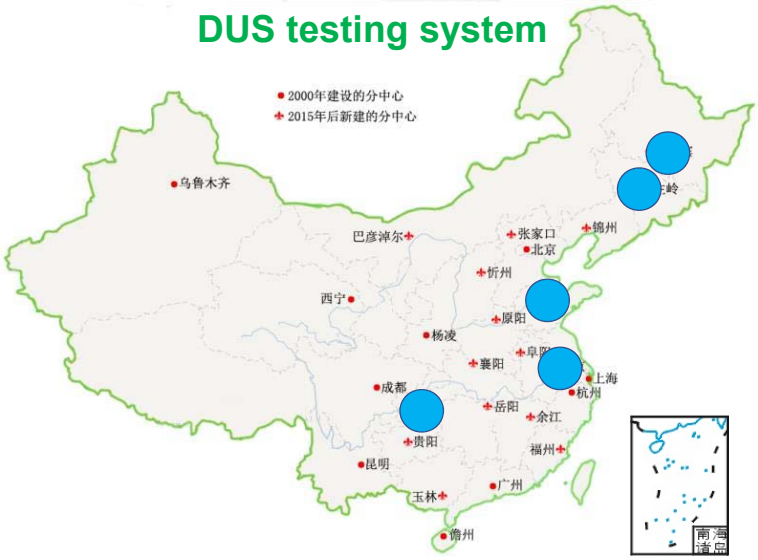
Numbers of varieties applying for Plant Variety Protection（Agriculture parts）



Applications by crop types



DUS testing system



Present: 1 headquarter+27 sub-centers+6 stations
Goal in 2020: 1 headquarter+29 sub-centers+28 stations
Mainly based on the agricultural institute or university

Molecular Lab of DUS Testing Center, MARA

ABI 3730 DNA analyzer

LGC SNP line

Affymetrix Gene Titan

Ion S5XL

ABI QuantStudio 7 Flex

LabChip GX Touch

Released MT Standards

Crops	No. of Chr (2n)	No. of Markers	date
Maize	20	40	2014
Rice	24	48	2014
Wheat	42	42	2013
Oil Seed	38	47	2013
Cotton	52	39	2013
Soybean	40	36	2013
Sorghum	20	40	2012
Barley	14	28	2013
Watermelon	22	28	2013
Cucumber	14	35	2013
Tomato	24	48	2013
Cabbage	18	20	2013
Pepper	24	22	2013
Chinese Cabbage	20	30	2013
Lily	24	20	2013
Apple	34/51	35	2013

VCK Database with DNA data

Crops	Number of Varieties (As of Oct 10, 2019)
Maize	11558
Wheat	6477
Rice	3086
Soybean	1686
Chinese cabbage	616
Pepper	407
Watermelon	190
Barley	163
Sorghum	334
French bean	161
Citrus	489

MT standards under development

33 MT standards are under development, e. g. Peach, Tea, Kiwifruit……

Citrus

French bean

Tomato

Red bean

Leaf mustard

Lettuce






Progress of SNP standards

Maize, Wheat, Rice, Soybean, Cotton, Vegetables and oilseed rape

Vegetables: Tomato, Pepper, Chinese cabbage, Cabbage, Watermelon and cucumber

Example 1: Wheat

2017 Jinan

Example 2: Chinese Cabbage

Ribenxiakang555

Nanfuxiaqiuwangdabaicai

Example 3: Pepper

China Accredited Seed Laboratory

中华人民共和国农作物种子质量检验机构

合格证书

CHINA ACCREDITED SEED LABORATORY CERTIFICATE

证书编号：（京）中种检字（2017）第001号

农业部植物新品种测试中心

依据《中华人民共和国种子法》和《农作物种子质量检验机构考核管理办法》的规定，经对你机构按照《农作物种子质量检验机构考核准则》进行条件审查和能力考评，考核合格，特发此证。

批准的检验项目范围见证书附表，准许在检验报告上标注CASL标志及证书编号。

有效日期：2022 年 9 月 11 日

发证日期：2017 年 9 月 12 日

GMO detection

Variety identification

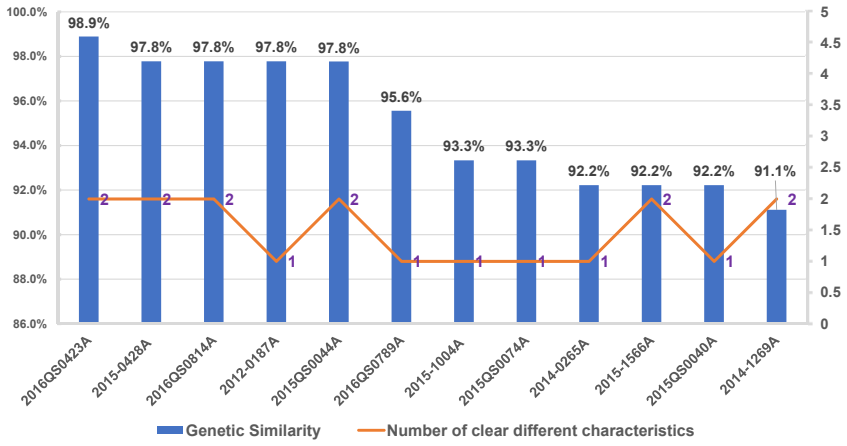
Chapter VI Seed Supervision and Management

Article 47 ……

The competent departments of agriculture and forestry may use the **rapid test methods** prescribed by the state to test the seed varieties of production and management, **and the test results can be used as the basis for administrative punishment**. If the person being inspected **disagrees with the test result, he may apply for re-examination, and the same test method shall not be used for re-examination.** If the test result is wrong or cause losses to the party, it shall be liable for compensation according to law.

Case: Jinhai 5 Hao ,2017-11-15

The burden of proof shall be borne by the party accused of the infringement.



Wheat, 2017

Leaf size and the degree of Green fruit are clearly distinguished

Future plan

1. New standards and database, e.g. Asexual propagated crops
2. Application of new methods, e.g. SNP, MNP
3. International cooperation/harmonization
4. Dissemination of DUS test and DNA methods

Thanks for your attention!

[End of Annex II and of document]