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MOLECULAR TECHNIQUES

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EXECUTIVE SUMMARY

1. This document presents proposals for the development of guidance on molecular markers in the examination of distinctness, uniformity and stability (DUS) and cooperation with international organizations, as follows:

- (i) Consider draft guidance on how to validate “characteristic-specific” molecular markers for DUS examination and standard template to describe their use to assess characteristics in Test Guidelines (see Annex to this document)
- (ii) Inviting France to coordinate work in UPOV for harmonizing terminology on molecular markers with the Organisation for Economic Co-operation and Development (OECD) and the International Seed Testing Association (ISTA)
- (iii) Surveying UPOV members to update the list of molecular makers used for each crop
- (iv) Cooperating with OECD and ISTA to develop common sets of molecular markers for variety identification

2. On “confidentiality and ownership of molecular information”, the Technical Committee (TC) is invited to note discussions at the Technical Working Parties (TWPs) in 2025 and consider organizing future discussions based on concrete cases and specific situations. The TC is invited to note that a “Policy on the status of plant material submitted for DUS testing purposes” was reported by the European Union and is available [online](#).

3. Discussions on molecular markers at the Technical Working Parties that took place since the 2024 UPOV sessions are reported for information purposes.

4. The following abbreviations are used in this document:

CAJ:	Administrative and Legal Committee
ISTA:	International Seed Testing Association
OECD:	Organization for Economic Co-operation and Development
TC:	Technical Committee
TWA:	Technical Working Party for Agricultural Crops
TWF:	Technical Working Party on Fruit Crops
TWM:	Technical Working Party on Testing Methods and Techniques
TWO:	Technical Working Party on Ornamental Plants and Forest Trees
TWPs:	Technical Working Parties
TWV:	Technical Working Party for Vegetables

5. The structure of this document is as follows:

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GUIDELINES FOR THE VALIDATION OF A NEW CHARACTERISTIC-SPECIFIC MOLECULAR MARKER PROTOCOL AS AN ALTERNATIVE METHOD FOR OBSERVATION

Background

6. The TC¹, at its sixtieth session, agreed to request the TWP^s, at their sessions in 2025, to consider the proposal for guidelines for the validation of new characteristic-specific molecular marker protocol for DUS examination.

Developments at the Technical Working Parties at their sessions in 2025

7. At their sessions in 2025, the TWO², TWM³, TWV⁴, TWA⁵, and TWF⁶ considered document TWP/9/4 and the proposed guidelines for validating assessment methods of characteristic-specific molecular markers for DUS examination, as presented by experts from the Netherlands (Kingdom of the).

Validation of Characteristic-Specific Molecular Markers for Test Guidelines.

8. The TWO noted that the proposed guidelines would be applied to validate molecular markers proposed as alternative methods for the assessment of individual characteristics in Test Guidelines.

9. The TWM noted that the proposed procedure related to one possible procedure for the validation of molecular markers and agreed that molecular markers could be validated through their publication in peer reviewed literature.

10. The TWA noted that the proposed guidance reported the experiences of France, Italy and the Netherlands (Kingdom of the) validating characteristic-specific molecular markers as alternative methods for the assessment of characteristics in Test Guidelines. The TWA agreed there should be flexibility for different validation types or procedures to be used as the basis for including molecular markers in Test Guidelines.

11. The TWF agreed with the procedure proposed to validate molecular markers developed by examination authorities for characteristic-specific molecular markers, to be used as alternative methods for the assessment of characteristics in Test Guidelines.

12. The TWM agreed that information in paragraphs 21 and 28 of document TWP/9/4 should be revised to clarify the validation methods. The TWM agreed that the text box for item 8 on the table should be amended to read as follows:

"In case the DNA marker test result does not confirm the declaration in the Technical Questionnaire, a field trial or bio-assay should be performed. ~~to assess the correctness of the declaration in the Technical Questionnaire.~~"

13. The TWV agreed the following amendments to the text on "Table 1":

- Items 1 and 2: to update reference to current version of the Tomato Test Guidelines (TG/44/12)
- Item 8: to read "[...] In case the DNA marker test result does not confirm the declaration in the Technical Questionnaire, a field trial or bio-assay should be performed ~~to assess the correctness of the declaration in the Technical Questionnaire.~~"

¹ TC, sixtieth session, held in Geneva, from October 21 to 22, 2024. See document TC/60/8 "Report", paragraphs 51

² TWO, fifty-seventh session, held in Roelofarendsveen, Kingdom of the Netherlands, from March 31 to April 3, 2025. See document TWO/57/10 "Report", paragraphs 22 to 23.

³ TWM, third session, held in Beijing, China, from April 28 to May 1, 2025. See document TWM/3/29 "Report", paragraphs 29 to 31.

⁴ TWV, fifty-ninth session, held via electronic means, from May 5 to 8, 2025. See document TWV/59/19 "Report", paragraphs 25 to 26.

⁵ TWA, fifty-fourth session, held Arusha, United Republic of Tanzania, from May 19 to 22, 2025. See document TWA/54/7 "Report", paragraphs 13 to 16.

⁶ TWF, fifty-sixth session, held Bursa, Türkiye, from June 23 to 26, 2025. See document TWF/56/7 "Report", paragraphs 32 to 36.

Trade Secret Molecular Markers

14. The TWO discussed, in the context of the development of UPOV Test Guidelines, matters to be considered on the use of molecular markers that might be trade secret (see Annex to this document, paragraph 31). The TWO noted that in such cases, the marker would not be described in the Test Guidelines, and permission would be required from the owner of the marker to be used by UPOV members. The TWO noted that molecular markers were most frequently provided by the breeders and was of the view that access for UPOV members to the markers which might be a trade secret would be important for international cooperation and exchange of DUS test reports.

15. The TWA and TWF discussed about access to the methods used in Test Guidelines in relation to the situation described in the Annex, paragraph 31, of the draft guidance (“trade secret protocol”). The TWA agreed that further consideration would be required in case a molecular marker with restricted access would be proposed for inclusion in Test Guidelines.

Standard Protocol for Characteristic-Specific Molecular Marker in Test Guidelines

16. The TWA and TWF agreed that the proposed standard protocol for characteristic assessment using molecular marker provided a suitable basis for harmonizing how information should be provided in Test Guidelines (see Annex, Section V “Standard Protocol for Characteristic-Specific Molecular Marker”).

Characteristics Developed Using New Breeding Techniques

17. The TWF discussed the use of molecular markers to assess characteristics developed using new breeding techniques. The TWF noted there was no experience reported on this matter and agreed to receive an update on experiences concerning the topic in future.

Proposal

18. Based on the comments from the TWPs, at their sessions in 2025, the TC may wish to consider the proposed guidance for the validation of molecular markers for inclusion in Test Guidelines as alternative methods of examination. The TC may wish to consider the inclusion of the proposed guidance in the UPOV series of documents specifying Test Guidelines’ Procedures (“TGP documents”).

19. The TC is invited to consider the proposed guidance for validation of molecular markers in Test Guidelines, as set out in the Annex to this document.

CONFIDENTIALITY AND OWNERSHIP OF MOLECULAR INFORMATION

Background

20. Since 2019, discussions on cooperation in relation to the use of molecular techniques include the request to address guidance on ownership and confidentiality of molecular information (see document [TC/55/7](#) “Molecular Techniques”). At its fifty-eighth session⁷, the TC noted the concerns expressed by breeders’ organizations that molecular information used during the examination of a variety should not be shared by the authority that received the application without the permission of the breeder. The TC invited members and observers to report on existing policies on confidentiality of molecular information at the TWPs, at their sessions in 2023⁷, 2024⁸ and 2025⁹.

⁷ TC, fifty-eighth session, held in Geneva, on October 24 to 25, 2022. See document TC/58/31 “Report”, paragraphs 48 to 50

⁸ TC, fifty-ninth session, held in Geneva, on October 23 to 24, 2023.

⁹ TC, sixtieth session, held in Geneva, on October 21 to 22, 2024.

Existing guidance

21. The following guidance is currently provided by UPOV on confidentiality of molecular information:

- (a) Document [UPOV/INF/15/4](#) “Guidance for Members of UPOV”, paragraph 38:

“38. As stated in Article 12, for the purposes of examination, the authority may require the breeder to furnish all the necessary information, documents or material. In that regard, authorities should give consideration to appropriate measures concerning confidentiality, for example in relation to pedigree information.”

- (b) Document [TGP/5, Section 1/3](#) “Model Administrative Agreement for International Cooperation in the Testing of Varieties”, Article 4:

“(1) The Authorities shall take all necessary steps to safeguard the rights of the applicant.”

“(2) Except with the specific authorization of the Receiving Authority and the applicant, the Executing Authority shall refrain from passing on to a third person any material, including DNA, or molecular information, of the varieties for which testing has been requested.”

Developments at the Technical Working Parties at their sessions in 2025

22. The TWO¹⁰, at its fifty-seventh session, agreed on the importance of utilizing DNA-based information for international cooperation in variety testing. The TWO considered how DNA-based information could be shared among UPOV members and noted the offer from breeders’ organizations for the joint development of molecular markers that would not reveal the breeding strategies of individual breeders.

23. The TWO noted existing guidance on confidentiality of molecular information in UPOV documents UPOV/INF/15 “Guidance for members of UPOV” and TGP/5, Section 1 “Model administrative agreement for international cooperation in the testing of varieties”. The TWO noted the proposal from the breeders’ organizations for considering the document “Policy on the status of plant material submitted for DUS testing purposes” developed by the European Union as example for the future development of a common model.

24. The TWM¹¹, at its third session, recalled UPOV guidance on confidentiality of molecular information provided in documents TGP/5, Section 1 “Model Administrative Agreement for International Cooperation in the Testing of Varieties” and INF/15 “Guidance for Members of UPOV”. The TWM noted that no reports on confidentiality of molecular information had been reported to the TWM and agreed on the importance of safeguarding the confidentiality of parent lines and hybrid formulas. The TWM noted that a similar discussion was being held at OECD.

25. The TWV¹², at its fifty-ninth session, noted that no reports on existing policies on confidentiality of molecular information had been reported in advance of the TWV session.

26. The TWV noted the report from Japan that was considering the use of DNA-based information as part of the information to be provided for plant variety protection. The TWV noted that Japan considered this information useful to support the exercise of breeders’ rights.

27. The TWV noted the concern expressed by the breeders’ organizations about revealing the sources of germplasm used by breeders in different breeding programs. The TWV agreed that breeders should be involved when selecting molecular markers for variety identification and managing variety collections, in particular when such information would be made publicly available.

28. The TWV recalled that the development of UPOV guidance was based on examples and experiences from UPOV members and observers. The TWV agreed that different approaches for discussion on confidentiality of molecular information should be considered, such as concrete cases and specific situations identified by the breeders’ organizations.

¹⁰ TWO, fifty-seventh session, held in Roelofarendsveen, Kingdom of the Netherlands, from March 31 to April 3, 2025. See document TWO/57/10 “Report”, paragraphs 24 to 27.

¹¹ TWM, third session, held in Beijing, China, from April 28 to May 1, 2025. See document TWM/3/29 “Report”, paragraphs 63 to 64.

¹² TWV, fifty-ninth session, held via electronic means, from May 5 to 8, 2025. See document TWV/59/19 “Report”, paragraphs 27 to 32.

29. The TWA¹³, at its fifty-fourth session, noted the reports from the European Union, Germany and the United Kingdom that their domestic policies on plant material and data used in DUS examination covered molecular information. The TWA noted that in the European Union, the technical questionnaire provided the opportunity for applicants to select information to be treated as confidential and that the policy on plant material in DUS testing was available online, as reported in document TWA/54/2 “Reports from members and observers” (see: <https://cpvo.europa.eu/en/cpvo-policy-status-plant-material-used-dus-testing-purposes>).

30. The TWA noted the reports from Brazil and Canada that no specific policy existed in those countries in relation to confidentiality of molecular information of plant material and data used in DUS examination. The TWA noted that Brazil and Canada did not request DNA based information from applicants.

31. The TWA discussed the possibility of issuing a survey for UPOV members to report on existing policies on confidentiality of molecular information. The TWA agreed that further discussion would be required on the scope and objectives of a survey, such as identifying relevant scenarios for increased engagement in discussions.

32. The TWF¹⁴, at its fifty-sixth session, noted that UPOV members and observers were invited to report examples of policies on confidentiality and access to molecular data at the TWP sessions in 2025.

33. *The TC is invited to:*

(a) note discussions on confidentiality and ownership of molecular information at the TWP sessions in 2025;

(b) note the “Policy on the status of plant material submitted for DUS testing purposes” reported by the European Union at the TWA, as provided in paragraph 29 of this document;

(c) consider inviting the TWPs to organize future discussions on confidentiality and ownership of molecular information based on concrete cases and specific situations; and

(d) consider inviting the TWPs to discuss cooperation on the use of molecular information, such as at the 2025 sessions of the TWO (joint development of molecular markers) and TWV (managing variety collections and variety identification).

COOPERATION BETWEEN INTERNATIONAL ORGANIZATIONS

Background

34. The TC¹⁵, at its fifty-seventh session, agreed to propose the following topics for a future joint UPOV/OECD/ISTA workshop:

- (i) providing information on the use of molecular techniques in each organization;
- (ii) procedure for approval of biochemical and molecular methods in each organization; and
- (iii) possibilities for harmonizing terms, definitions and methods between UPOV, OECD and ISTA.

¹³ TWA, fifty-fourth session, held Arusha, United Republic of Tanzania, from May 19 to 22, 2025. See document TWA/54/7 “Report”, paragraphs 17 to 21.

¹⁴ TWF, fifty-sixth session, held Bursa, Türkiye, from June 23 to 26, 2025. See document TWF/56/7 “Report”, paragraphs 37 to 38.

¹⁵ TC, fifty-seventh session, held in Geneva, on October 25 to 26, 2021.

Harmonizing terms, definitions and methods between UPOV, OECD and ISTA

35. The TWM¹⁶, at its third session, considered possible joint activities with OECD and ISTA and the possible harmonization of terms, definitions and methods in relation to molecular techniques. The TWM agreed to invite the expert from France to coordinate discussions to organize relevant information on terms and definitions. The TWM noted the expression of interest of the experts from Argentina, China, Germany, Netherlands (Kingdom of the), United Kingdom, CIOPORA and ISF to contribute to the exercise.

36. The TWM noted the report from the representative of OECD that the OECD Seed Schemes had already endorsed the collaboration with UPOV for possible harmonization of definitions and terms.

37. *The TC is invited to:*

(a) consider inviting France, in collaboration with Argentina, China, Germany, Netherlands (Kingdom of the), United Kingdom, CIOPORA and ISF, to organize information on terms and definitions on molecular techniques used in UPOV, for consideration by the TWM and the TC in 2026; and

(b) note that the OECD Seed Schemes endorsed the collaboration with UPOV for possible harmonization of definitions and terms.

Updating the list of molecular makers used per crop

38. The TWM recalled that the outcomes of the survey of UPOV members on the use of molecular markers per crop was available as a spreadsheet at the webpage of the Technical Committee, at its fifty-eight session (see: https://www.upov.int/meetings/en/doc_details.jsp?meeting_id=67786&doc_id=586962).

39. The TWM¹⁷ welcomed the proposal from the Netherlands (Kingdom of the) to coordinate the updating of the list of molecular markers used per crop, that had been reported to the Technical Committee, at its fifty-eight session.

40. The TC is invited to consider inviting the Netherlands (Kingdom of the) to coordinate the updating of the list of molecular markers used per crop developed by UPOV and available at the webpage of the fifty-eighth session of the TC.

Common sets of molecular markers for variety identification*Horizon scanning*

41. The UPOV horizon scanning exercise summarized in UPOV's Strategic Business Plan (SBP) for 2026-2029 (document C/59/14) has identified the increasing reliance on DNA analysis for variety identification purposes. It has also made a call for action for UPOV to develop standards in this field and explore means for data management and collaborations on data science activities.

Developments at the Technical Working Party on Testing Methods and Techniques

42. The TWM¹⁸ discussed the establishment of common sets of molecular markers for variety identification and agreed to invite UPOV, OECD and ISTA to further consider the challenges and opportunities of this initiative, such as crop(s), scale of harmonization (e.g. regional, global); and molecular marker-related aspects. The TWM agreed that working with breeders could facilitate selecting marker sets representing those breeding programs.

¹⁶ TWM, third session, held in Beijing, China, from April 28 to May 1, 2025. See document TWM/3/29 "Report", paragraphs 20 to 28.

¹⁷ TWM, third session, held in Beijing, China, from April 28 to May 1, 2025. See document TWM/3/29 "Report", paragraphs 35.

¹⁸ TWM, third session, held in Beijing, China, from April 28 to May 1, 2025. See document TWM/3/29 "Report", paragraph 28.

43. Following the TWM session, the Annual Meeting of the OECD Seed Schemes, approved for the OECD Seed Schemes to collaborate with ISTA and UPOV to start working on the establishment of common sets of molecular markers for variety identification. The OECD Seed Schemes agreed to invite National Designated Authorities (NDAs) to provide the names of experts to start working on this topic.¹⁹

44. *The TC and the CAJ are invited to:*

(a) *endorse the collaboration with OECD and ISTA to establish common sets of molecular markers for variety identification;*

(b) *consider whether to select an expert and/or interested experts to coordinate work on the topic; and*

(c) *note that OECD approved the start of work to establish common sets of molecular markers for variety identification, in collaboration with ISTA and UPOV.*

Information on the use of molecular techniques in each organization: possible joint meeting

45. The TWM discussed the possibility of a joint meeting with participants from the TWM, OECD Seed Schemes and ISTA Variety Committee to discuss cooperation on the use of molecular markers for the purposes of each organization. The TWM agreed that organizing a joint meeting with experts from the three organizations would require specific arrangements and should be further discussed by UPOV, OECD and ISTA.

46. The TC and the CAJ may wish to endorse the organization of a joint meeting with participants from the UPOV, OECD Seed Schemes and ISTA Variety Committee to discuss cooperation on the use of molecular markers for the purposes of each organization. In case positive, the arrangements for the joint meeting would be proposed for consideration by OECD and ISTA. Future hosts of TWM sessions could also be consulted in this regard.

47. *The TC and the CAJ are invited to endorse the organization of a joint meeting with participants from the UPOV, OECD Seed Schemes and ISTA Variety Committee to discuss cooperation on the use of molecular markers for the purposes of each organization.*

MATTERS FOR INFORMATION

48. The TWM held its third session in Beijing, China from April 28 to May 1, 2025. The following sections report developments on molecular techniques.

Latest developments in molecular techniques and bioinformatics

Data science activities at Naktuinbouw towards genotyping and phenotyping: an update

49. The TWM received a presentation from Ms. Sanchari Sircar (Netherlands (Kingdom of the)) on “Data science activities at Naktuinbouw towards genotyping and phenotyping: an update”, a copy of which is provided in document TWM/3/16.

50. The TWM noted the development of software at Naktuinbouw and the invitation for collaboration on data science activities, including image analysis and phenotyping, workflow developments, artificial intelligence and other collaborative efforts.

¹⁹ OECD Seed Schemes Annual Meeting, June 12-13, 2025. See document “Confirmation of outcomes”.

Cooperation between international organizations*ISTA*

51. The TWM received a presentation from Ms. Ana Vicario, International Seed Testing Association (ISTA), on “ISTA update on the use of techniques for variety identification and verification”, a copy of which is provided in document TWM/3/25.

52. The TWM noted that the markers selected for detecting perennial types in annual ryegrass were not necessarily associated with morphological characteristics and were based on varieties from different countries. The TWM noted that the markers identified in the project would be published in the ISTA rules.

53. The TWM noted that the neural network used in support of variety identification was a proprietary software.

OECD

54. The TWM received a presentation from Mr. Csaba Gaspar, Organisation for Economic Co-operation and Development (OECD), on “Latest developments in the application of BMT under the OECD Seed Schemes”, a copy of which is provided in document TWM/3/26.

Report of work on molecular techniques in relation to DUS examination*Latest developments in characteristic-specific molecular markers at Naktuinbouw: a call for knowledge exchange*

55. The TWM received a presentation from Ms. Claire Kamei (Netherlands (Kingdom of the)) on “Latest developments in characteristic-specific molecular markers at Naktuinbouw: a call for knowledge exchange”, a copy of which is provided in document TWM/3/7.

56. The TWM noted that Naktuinbouw was initiating a project for the selection of molecular markers for lettuce and that interested experts should contact the expert from the Netherlands (Kingdom of the) for possible partnerships.

57. The TWM agreed that organizations should consider pooling resources in support of common projects. The TWM considered options to make available information about projects developed by UPOV members and observers and agreed they could be reported before each TWM session for inclusion in document TWM/3/2 “Reports on Developments in Plant Variety Protection from Members and Observers”.

58. The TWM welcomed the proposal from the Netherlands (Kingdom of the) to lead the updating of the list of molecular markers used per crop, that had been reported to the Technical Committee, at its fifty-eight session (available at: https://www.upov.int/meetings/en/doc_details.jsp?meeting_id=67786&doc_id=586962).

The use of biomolecular technology in DUS testing - a case study on barley

59. The TWM received a presentation from Ms. Vanessa MacMillan (United Kingdom) on “The use of biomolecular technology in DUS testing - a case study on barley”, a copy of which is provided in document TWM/3/20.

60. The TWM noted the report provided in the document and invited the expert from the United Kingdom to report progress at the fourth session of the TWM.

Artificial Intelligence and molecular markers in soft fruit: a proof of concept

61. The TWM received a presentation from Ms. Margaret Wallace (United Kingdom) on “Artificial Intelligence and molecular markers in soft fruit: a proof of concept”, a copy of which is provided in document TWM/3/24.

62. The TWM noted progress in the genetic prediction of morphological characteristics such as the presence of spines in Raspberry. The TWM discussed factors relating to the genetic prediction of morphological characteristics as they related to the results demonstrated in the proof of concept study.

Can better understanding of the genetic architecture of wheat DUS characteristics help streamline the DUS processes?

63. The TWM received a presentation from Ms. Camila Zanella (United Kingdom) on “Can better understanding of the genetic architecture of wheat DUS characteristics help streamline the DUS processes?”, a copy of which is provided in document TWM/3/22.

64. The TWM considered the requirements for implementing molecular markers in routine variety examination and agreed that they should at the same time increase efficiency for the examination authority and benefit the applicants.

Genomic prediction for variety collection management wheat

65. The TWM received a presentation from Mr. Adrian Roberts (United Kingdom), on “Genomic prediction for variety collection management wheat”, a copy of which is provided in document TWM/3/6.

66. The TWM noted that adjustments were required for the method to work with notes (ordinal data) instead of actual measurements and invited the expert from the United Kingdom to report progress at the fourth session of the TWM.

COYD-GP enhanced distinctness criterion for cross-pollinated agricultural crops

67. The TWM received a presentation from Mr. Adrian Roberts (United Kingdom), on “COYD-GP enhanced distinctness criterion for cross-pollinated agricultural crops”, a copy of which is provided in document TWM/3/4.

68. The TWM noted that the increased efficiency of the new method COYD-GP for distinctness assessments had been calculated for each characteristic and agreed that further investigation would be required on the overall efficiency gain. The TWM invited the expert from the United Kingdom to report developments at the fourth session of the TWM.

Community Plant Variety Office (CPVO) R&D activities

69. The TWM received a presentation from Ms. Cecile Collonnier, Community Plant Variety Office (CPVO), on “CPVO R&D activities”, a copy of which is provided in document TWM/3/15.

70. The TWM noted the report on recently concluded and ongoing projects co-funded by the CPVO. The TWM noted that the molecular markers selected under the projects were publicly available and noted the offer from China to exchange a selection of KASP markers.

Methods for analysis of molecular data, management of databases and exchange of data and material

Exploiting crop haplotype-tag polymorphisms marker for pedigree identification

71. The TWM received a presentation from Mr. Yikun Zhao, China, on “Exploiting crop haplotype-tag polymorphisms (HTP) marker for pedigree identification”, a copy of which is provided in document TWM/3/10.

72. The TWM discussed the usefulness of HTP makers for pedigree identification in maize three-way hybrids and its possible use for soybeans. The TWM discussed the statistical methods to assess confidence of the method and noted the correct identification of 94% of samples in the tests performed. The TWM noted that HTP makers could possibly be used for assessing essentially derived varieties (EDVs). The TWM agreed to invite the expert from China to report developments at its fourth session.

PAD – an algorithm for progeny-ancestor detection based on genetic profiles

73. The TWM received a presentation from Mr. Emerson Limberger, International Seed Federation (ISF), on “PAD – an algorithm for progeny-ancestor detection based on genetic profiles”, a copy of which is provided in document TWM/3/17.

74. The TWM noted that MNP markers would provide better results, but in the absence of MNP markers, genetic tags based on recombination blocks could be used as alternative, although further testing was necessary. The TWM noted that a test version of the algorithm would be made available for interested experts.

DurdusTools: Current state and use in DUS-testing

75. The TWM received a presentation from Ms. Alexandra Ribarits (Austria), on “DurdusTools: Current state and use in DUS-testing”, a copy of which is provided in document TWM/3/21.

76. The TWM noted the use of DurdusTools calculating genetic distances in support of routine DUS examination of the participation authorities since 2024. The TWM noted that the participating authorities covered the operational costs, including database maintenance and molecular data generation.

Development of DUS phenotyping tools for and with examination offices: experience gained

77. The TWM received a presentation from Mr. Joseph Peller (Netherlands (Kingdom of the)), on “Development of DUS phenotyping tools for and with examination offices: experience gained”, a copy of which is provided in document TWM/3/27.

78. The TWM noted the availability of a mobile phone application prototype to assess volume and shape ratios of fruits, for images captured from a top down perspective. The TWM noted that the programming code for the application was open source and available at GitHub. The TWM noted the invitation for collaboration to further develop the application, in particular for stabilizing the mobile phone interface. The application and tutorial are available at: https://play.google.com/store/apps/details?id=com.wur.invite.morph_app&hl=en-US).

79. The TWM agreed on the importance of applications for hand-held devices in support of increased efficiency in DUS examination.

Phenotyping concept for strengthening the plant variety protection chain via combined use of IA&AI

80. The TWM received a presentation from Mr. Zsolt Szani, Hungary on “Phenotyping concept for strengthening the plant variety protection chain via combined use of image analysis and artificial intelligence (IA&AI)”, a copy of which is provided in document TWM/3/28.

81. The TWM considered the use of algorithms for image analysis and agreed they should be described and validated. The TWM agreed that the introduction of phenotyping tools in variety examination requires sufficient amount of variety data for training the algorithms and validation of the analysis generated.

Use of DNA databases at Naktuinbouw to improve DUS work

82. The TWM received a presentation from Ms. Cécile Marchenay (Netherlands (Kingdom of the)) on “Use of DNA databases at Naktuinbouw to improve DUS work”, a copy of which is provided in document TWM/3/8.

83. The TWM discussed challenges and opportunities on the use of DNA-based information as the basis to optimize variety collections and the organization of growing trials. The TWM discussed the use of DNA-based information to reduce the number of growing cycles for crops that would normally be examined in two growing trials.

Shared molecular database

84. The TWM received a presentation from Mr. Rene Mathis (France) on “Shared molecular database”, a copy of which is provided in document TWM/3/23.

85. The TWM agreed on the usefulness of shared databases and noted the plans for shared databases in the European Union.

The use of molecular techniques in the assessment of essential derivation*Exploration of identification techniques based on SNP markers for essentially derived varieties of wheat*

86. The TWM received a presentation from Ms. Binshuang Pang (China) on “Exploration of identification techniques based on SNP markers for essentially derived varieties of wheat”, a copy of which is provided in document TWM/3/11.

87. The TWM noted the method for establishing a 92% threshold of predominant derivation using at least 20,000 SNPs and commonly known essentially derived varieties (EDV) as the basis for the analysis.

88. The TWM agreed that the variety selection method utilized and its pedigree were important elements for the assessment of essential derivation. The TWM recalled the UPOV guidance in document UPOV/EXN/EDV/3 that a high degree of similarity alone did not automatically mean that a variety had been predominantly derived, such as in the case of convergent breeding.

89. The TWM noted that the method described in the presentation was a recalibration using SNPs of a previously established threshold using SSR markers.

Essentially derived varieties (EDV) threshold development in soybeans

90. The TWM received a presentation from Mr. Barry Nelson, International Seed Federation (ISF), on “Essentially derived varieties (EDV) threshold development in soybeans”, a copy of which is provided in document TWM/3/9.

91. The TWM noted that the preliminary threshold would be evaluated by breeders involved in the study according to their current soybean development programs; if the threshold was agreed upon, it would be shared with relevant seed associations for agreement and potential adoption.

92. The TWM agreed on the importance of breeders’ contributions to determining thresholds and avoiding disputes on EDVs. The TWM agreed that implementing a threshold would require looking at variety pedigrees and how to assess remaining criteria for determining essential derivation.

The use of molecular techniques for enforcement

Use of DNA techniques for plant variety right (PBR) enforcement in Peru

93. The TWM received a presentation from Mr. Diego F. Ortega Sanabria (Peru) on “Use of DNA techniques for plant variety right (PBR) enforcement in Peru”, a copy of which is provided in document TWM/3/3.

94. The TWM noted the procedures in Peru for field inspections of infringement cases, including the role of the administrative authority to conduct field inspections and the existence of guidelines for DNA-based information. The TWM noted that in Peru the plaintiff should demonstrate the specificity of the markers to be used identifying the protected variety.

95. The TWM noted the challenges reported in relation to enforcement on exported fruits due to the amount of time required for variety identification. The TWM agreed that it was important to strengthen cooperation among authorities in UPOV members on enforcement matters.

Use of molecular markers as a tool to enforce plant variety right (PBR) in soybean in Uruguay

96. The TWM received a presentation from Ms. Vanessa Sosa and Ms. Pilar Zorilla (Uruguay) and Mr. Diego Risso (Seed Association of the Americas) on “Use of molecular markers as a tool to enforce plant variety right (PBR) in soybean in Uruguay”, a copy of which would be provided as an addendum to document TWM/3/18.

97. The TWM noted that in Uruguay the breeders’ association and the National Seeds Institute conducted field inspections. The TWM noted that the procedure for variety identification could take up to two days, in some cases. The TWM noted that infringement fines in Uruguay were based on the value of the harvested material and considered an effective measure.

98. The TWM noted that image analysis was also used for variety identification using seeds of protected varieties.

[Annex follows]

GUIDELINES FOR THE VALIDATION OF A NEW CHARACTERISTIC-SPECIFIC MOLECULAR MARKER PROTOCOL AS AN ALTERNATIVE METHOD FOR OBSERVATION

Document prepared by experts from France, Italy and the Kingdom of the Netherlands

Disclaimer: this document does not represent UPOV policies or guidance

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ASSOCIATED DOCUMENTS

- TG/1/3: General Introduction to the Examination of Distinctness, Uniformity and Stability and the Development of Harmonized Descriptions of new Varieties of Plants
- TG/44: Guidelines for the conduct of tests for distinctness, uniformity and stability for Tomato
- TGP/9: Examining distinctness
- TGP/10: Examining uniformity
- TGP/12: Guidance on Certain Physiological Characteristics
- TGP/15: Guidance on the Use of Biochemical and Molecular Markers in the Examination of Distinctness, Uniformity and Stability (DUS)
- UPOV/INF/17 Guidelines for DNA-Profiling: Molecular Marker Selection and Database Construction
- UPOV/INF/18 Possible use of Molecular Markers in the Examination of Distinctness, Uniformity and Stability (DUS)
- TWV/54/7 + Add Use of molecular techniques in DUS examination

I. OBJECTIVES OF THESE GUIDELINES

1. The purpose of these guidelines is to elaborate the principles contained in the General Introduction (document TG/1/3), and its associated TGP documents, into detailed practical guidance for the harmonized validation of a new method based on characteristic-specific molecular marker before its use as an alternative test. Performance criteria required for the validation are described and guidance on their assessment is given. These guidelines also describe a standard protocol with mandatory and optional chapters. Survey after acceptance is also described.
2. If a different technique is used, the laboratory must validate its method in comparison to the reference method (to show that the alternative technique gives the same results).

II. SCOPE OF THESE GUIDELINES

All crops

Characteristic-Specific Molecular Markers

For the examination of Distinctness, Uniformity, and Stability (DUS).

III. PERFORMANCE CRITERIA FOR A NEW MOLECULAR MARKER BASED PROTOCOL

Specificity

Definition

3. Correlation between the genotype and the phenotype, *i.e.* reliability of the link between the marker and the characteristic.

Requirement

4. In principle 100% of correlation between the genotype and the phenotype. If the correlation is less than 100% a follow-up test(s) should be performed to ensure the reliability of the results. A decision rule can be used in that case. Less than 100% correlation can be caused by other genetics.

How to evaluate it?

5. Number of varieties: To start the marker selection process an appropriate number of varieties (development set) is needed to reflect at the most the diversity observed within the group/crop/species/type for which the markers are intended to be discriminative.
6. Varieties should represent the different states of expression (if known varieties with heterozygous and homozygous state), coming from different plant breeders, with different genetic background of the characteristic and different types. Well phenotypically characterized varieties for the trait of interest should be used when available.
7. Number of plants per variety: At least one plant per variety if available varieties are phenotypically well characterized. If not, the number of plants should be the same as for the morphological observation described in the UPOV Test Guidelines.

8. The specificity can be assessed within one laboratory.

Sensitivity and limit of detection

Definition

9. The limit of detection is defined as the minimal quantity of the target that can be reliably detected.
10. In case of analyses performed on bulk samples (*e.g.* pool of different plants of the same variety) the sensitivity is critical and must be assessed. For the use on individual plants, the quantity of the target is not critical and this performance criterium is optional.

Requirement

11. In the case of the pool, the requirement would be to detect at least one off-type in the pool.

How to evaluate it?

12. To use artificial samples by mixing one off-type to a pool to check the sensitivity of the detection.

Repeatability

Definition (based on ISO 16 577:2016; reference to UPOV/INF/17)

13. *“Repeatability; where identical test results are obtained with the same method, on identical test items, in the same laboratory, by the same operator, using the same equipment within short intervals of time.”*

14. For qualitative methods, accordance is equivalent to the repeatability of quantitative methods (Langton *et al.*, 2002).

Requirement

15. Ideally 100%, a performance $\geq 90\%$ is generally accepted. If the repeatability of the reference method is published the repeatability of the alternative method should be at least equivalent.

How to evaluate it?

16. The repeatability can be evaluated within one laboratory.
17. At least three technical replicates drawn from a same plant (three independent DNA extractions). To include at least all expected types of genotype.

Reproducibility

Definition (based on ISO 16 577:2016; reference to UPOV/INF/17)

18. *“Reproducibility; where test results are obtained with the same method, on identical test items, within the same laboratory or between different laboratories, with different operators, using different equipment” at different times.*

19. For qualitative methods, concordance is equivalent to the reproducibility of quantitative methods (Langton *et al.*, 2002).

Requirement

20. Ideally 100%, a performance $\geq 90\%$ is generally accepted. If the reproducibility of the reference method is published the reproducibility of the alternative method should be at least equivalent.

How to evaluate it?

21. Reproducibility should be assessed between different laboratories by an interlaboratory validation study (Ring-test) with coded samples of known genotypes. All expected types of genotype should be included.

22. The ring-test should involve at least, three different laboratories including at least two different examination offices (e.g. in INVITE EU-project 817970 DOI [10.3030/817970](https://doi.org/10.3030/817970), 3 examination offices were involved in the validation test). If possible, experienced laboratories familiar with the species and the technique should be involved. If not, a training can be organized ahead of the ring-test with un-coded samples. Laboratories can participate in a ring-test on voluntary basis. In case there are no volunteers, then an intra-laboratory reproducibility assesment will be possible with different operators.

23. All laboratories must follow the protocol to be validated. In the protocol compulsory and optional parts can be defined by the validation team. Laboratories can participate in a ring-test on voluntary basis. In case there are no volunteers, the reproducibility can be determined within one laboratory.

24. Number of varieties: To include at least all expected types of genotype.

25. Guidelines/Norms on interlaboratory studies can be followed: ISO 13495 *Foodstuffs - Principles of selection and criteria of validation for varietal identification methods using specific nucleic acid*, ISO 17043 *Conformity assessment - General requirements for proficiency testing*, EPPO pm7-122-2 *Guidelines for the organization of interlaboratory comparisons by plant pest diagnostic laboratories*, ISTA TCOM-P-10-*Validation of seed health methods and organization and analysis of interlaboratory comparative tests (CT)*... The validation team can cite the followed guidelines in its report.

Robustness

Definition (based on ISO 16 577:2016; reference to UPOV/INF/17)

26. “Robustness; a measure of its capacity to remain unaffected by small, but deliberate deviations from the experimental conditions described in the procedure parameters and provides an indication of its reliability during normal usage” (e.g. change of DNA extraction method or change of real time machine).

Requirement

27. Ideally 100%, if less that means that the method is not robust to a change of one parameter and this should be indicated in the protocol as a mandatory step (e.g. a change of a mastermix that would be critical).

How to evaluate it?

28. It is optional to assess, and robustness is evaluated partially during the ring test (reproducibility), (different laboratories, equipment, machinery, ~~persons~~, etc.).

IV. VALIDATION REPORT

29. The validation report and results must be peer-reviewed by two (preferably 3 if the reproducibility was done within one laboratory) of the responsible bodies. Reviewing is on voluntary basis but preferably performed by laboratory familiar with the species and the method.

30. During the reviewing process, the reviewers can require extra validation data in concertation with the validation team.

Content of the validation report

- Raw data generated during the different steps of the validation process
- Detail protocol with optional and compulsory steps defined
- Performance criteria assessment
- Conclusion

Publicity

31. The validation report should be available upon request. In the new protocol the validation process should be mentioned with the contact examination office. In some particular cases, e.g. a “trade secret protocol” (cytoplasmic male sterility in cabbage), the protocol and the validation report could ~~not~~ be only shared ~~outside of the~~ between examination offices.

V. STANDARD PROTOCOL FOR CHARACTERISTIC-SPECIFIC MOLECULAR MARKER

32. Compulsory elements are indicated in the column “essential information”, the other elements may be used depending on the characteristic test protocol. If a laboratory wants to adapt/modify/change a mandatory chapter or element of a mandatory chapter it must validate its method in comparison to the reference method (to show that you obtain the same results as the published method).

Table 1: Standard characteristic-specific molecular marker protocol (Modifications are highlighted in grey)

Chapter	Elements in a Standard characteristic-specific molecular marker protocol	Example	Essential information for harmonization	Remark
1	characteristic	Resistance to Tomato mosaic virus (ToMV) <i>See TG/44/1241/rev3 – Ad 51: ii DNA marker test</i>	YES	
2	Genes and alleles	<i>See TG/44/1241/rev3 – Ad 51: ii DNA marker test add 2</i>	YES	Need to avoid dominant marker or presence/absence marker otherwise the robustness should be assessed
2.1	Targeted gene(s)	Resistance Gene <i>Tm2</i> Arens, P. et al (2010)	YES	a) file(s) containing the DNA sequence information (order of nucleotides) b) reference to DNA information in public databases (like GeneBank) c) reference to (scientific) publications in which the DNA sequence information of the states of expression of the characteristic is revealed. d) reference to a particular position on the published reference genome version.
2.2	Allele corresponding to expression state 1	<i>tm2</i> Arens, P. et al (2010)	YES	a) file(s) containing the DNA sequence information (order of nucleotides) b) reference to DNA information in public databases (like GeneBank) c) reference to (scientific) publications in which the DNA sequence information of the states of expression of the characteristic is revealed. d) reference to a particular position on the published reference genome version in combination with the SNP or INDEL that is responsible for the state of expression.
2.3	Allele corresponding to expression state n	<i>Tm2 and Tm2²</i> Arens, P. et al (2010)	YES	a) file(s) containing the DNA sequence information (order of nucleotides) b) reference to DNA information in public databases (like GeneBank) c) reference to (scientific) publications in which the DNA sequence information of the states of expression of the characteristic is revealed. d) reference to a particular position on the published reference genome version in combination with the SNP or INDEL that is responsible for the state of expression.
3	Primers (and probes)	<i>See TG/44/11/rev3 – Ad 51: ii DNA marker test add 3, 3.1 and 3.2</i>	YES	Primer and probe sequences, reference to accessions and sequences in public databases (Genebank numbers), literature
3.1	Primers (and probes) to detect allele '9'		YES	Primer Sequences corresponding to allele(s) for expression '9' (resistance)
3.2	Primers (and probes) to detect allele '1'		YES	Primer Sequences corresponding to allele(s) for expression '1' (susceptibility)
3.3	Primers (and probes) to detect allele 'x'		YES	Primer Sequences corresponding to allele(s) for expression 'x'
4	Format of the test			

Chapter	Elements in a Standard characteristic-specific molecular marker protocol	Example	Essential information for harmonization	Remark
4.1	Number of plants per genotype	≥20	YES	A minimal number of individual plants required: the test for the marker is conducted on the same number of individual plants, with the same criteria for distinctness, uniformity and stability as for the examination of the characteristic by an observation assay (documents TGP/9 and TGP/10)
4.2	Control varieties	<i>See TG/44/11/rev3 – Ad 51: ii DNA marker test add 4.2</i>	YES	Control varieties (same as in observation assay) as standards representing all relevant combination of alleles. For example homozygous for Allele corresponding to expression state 9 (present), homozygous for allele corresponding to expression state 1 (absent) and heterozygous (both alleles are present in a diploid) corresponding to either resistant, susceptibility or intermediate resistance of the variety (depending on gene function; dominant - recessive). DNA controls can be directly used.
4.3	Process controls	<i>e.g. buffer used for extraction; a marker targeting the cytochrome oxidase gene as an internal amplification marker</i>	YES	<ul style="list-style-type: none"> a) Negative process control(s) b) Positive DNA control(s) that can be the control varieties c) Internal amplification control in case of a presence/absence marker
5	Preparations	<i>e.g. Sampling of seedlings 4 days old followed by DNA extraction using CTAB method</i>	NO	Depending on the method used. Not in the Test Guideline. Detailed protocol(s) can be provided as an example in annex or available on request from the organization that developed the marker
6	Technique of the method	<i>e.g. conventional PCR, TETRA-ARMS, qPCR, KASP, amplicon sequencing</i> <i>See TG/44/11/rev3 – Ad 51: ii DNA marker test add 6</i>	YES	.
6.1	Particular conditions	<i>e.g. PCR protocol describing primer, enzyme, dNTP concentrations, PCR cycle scheme</i>	NO	Depending on the method used. Not in the Test Guidelines. Detailed protocol(s) can be provided as an example in annex or available on request from the institute that developed the marker
6.2	Particular hardware or infrastructure	<i>e.g. machines, commercial kits, manufactures of components, lot numbers of chemicals</i>	NO	Depending on the method used. Not in the Test Guidelines. Detailed protocol(s) can be provided as an example in annex or available on request from the institute that developed the marker
7	Observations	<i>e.g. Bands on agarose gel (conventional PCR), Ct values (qPCR) Variant call based on sequencing reads</i>	NO	Depending on the method used. Not in the Test Guidelines. Detailed protocol(s) can be provided as an example in annex or available on request from the institute that developed the marker

Chapter	Elements in a Standard characteristic-specific molecular marker protocol	Example	Essential information for harmonization	Remark
7.1	Validity of the results	e.g. for qPCR, Check for typical exponential amplification curves. Check if the controls are as expected (negative controls = no signal; positive controls = shows expected signals for all fluorophores).	YES	Depending on the method used.
8	Interpretation of the test results	See TG/44/11/rev3 – Ad 51: <i>ii DNA marker test add 8</i>	YES	Relation between alleles and expressions (with its notes) In case the DNA marker test result does not confirm the declaration in the Technical Questionnaire, a field trial or bio-assay should be performed to assess the correctness of the declaration in the Technical Questionnaire.
9	Validation of the method,	This protocol was validated by a ring-test with different laboratories	YES	(e.g. interlaboratory Comparative Test INVITE 2024 817970 DOI 10.3030/817970)
9.1	Contact Examination Office	e.g. Naktuinbouw	YES	Contact of the institute that developed this protocol, Name of the service.

VI. FOLLOW-UP SURVEY AFTER APPROVAL

33. Validation of the marker is not fixed as new genetics can arise from the market. This is a continuous evaluation process. Specificity should be re-assessed after validation acceptance using parallel testing (marker test and bioassay) at least during the first year with observation method.

34. After the first year of acceptance of the protocol, morphological checks on about 10% of the new varieties must be performed.

VII. LITERATURE

Arens P., Mansilla C., Deinum D., Cavellini L., Moretti A., Rolland S., van der Schoot H., Calvache D., Ponz F., Collonnier C., Mathis R., Smilde D., Caranta C.; Vosman B., 2010: Development and evaluation of robust molecular markers linked to disease resistance in tomato for distinctness, uniformity and stability testing. Theoretical and applied genetics 120(3). pp. 655-64

Langton, S.D., Chevennement, R., Nagelkerke, N. and Lombard, B. (2002). Analysing collaborative trials for qualitative microbiological methods: concordance and concordance. International Journal of Food Microbiology, 79, 175-181

[End of Annex and of document]