UPOV
International Union for the Protection of New Varieties of Plants

E

Technical Committee
Sixtieth Session
Geneva, October 21 and 22, 2024

Administrative and Legal Committee
Eighty-First Session
Geneva, October 23, 2024

SESSIONS/2024/6

Original: English
Date: September 25, 2024

MOLECULAR TECHNIQUES

Document prepared by the Office of the Union

Disclaimer: this document does not represent UPOV policies or guidance

EXECUTIVE SUMMARY

1. The purpose of this document is to report to the Technical Committee (TC) and the Administrative and Legal Committee (CAJ) on developments concerning molecular techniques.

2. The TC is invited to:

(a) request the Technical Working Parties (TWPs), at their sessions in 2025, to consider the proposal for guidelines for the validation of new characteristic-specific molecular marker protocol for DUS examination, as provided in the Annex to this document;

(b) note the request from breeders' organizations for the development of guidance in UPOV on confidentiality of molecular data and the offer to propose a draft model agreement template, to be presented at the third session of the Technical Working Party on Testing Methods and Techniques (TWM); and

(c) note the matters for information provided in this document.

3. 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

4. The structure of this document is as follows:

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

5. The TWM[1], at its second session, received a presentation from Ms. Amandine LeVan (France) on “Guidelines for the validation of a new characteristic-specific molecular marker protocol for DUS studies as an alternative method for observation”, a copy of which is provided in document TWM/2/17. The TWM noted that the proposals made during the presentation would be considered by the TWV and reported to the TC, at their sessions in 2024.

6. The TWV[2], at its fifty-eighth session, considered document TWV/58/9, presented by an expert from the Netherlands (Kingdom of). Document TWV/58/9 is reproduced in the Annex to this document.

7. The TWV agreed that guidance on the assessment of characteristics using the molecular markers presented in Test Guidelines would benefit from international harmonization.

8. The TWV agreed to propose the deletion of the last sentence in paragraph 5 and the inclusion of reference to the respective UPOV guidance applicable to the ISO standards mentioned.

9. The TWV agreed to propose amending the information provided in the protocol table, item 8, to clarify that “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.”

10. The TWV noted that characteristic-specific molecular markers could be used by the breeders and agreed that they were entitled to inform the examiner on the method of assessment used to assess characteristics in the Technical Questionnaire, in cases where a molecular marker was available as alternative to the one indicated in the Technical Questionnaire.

11. The TC is invited to request the TWPs, at their sessions in 2025, to consider the proposal for guidelines for the validation of new characteristic-specific molecular marker protocol for DUS examination, as provided in the Annex to this document.

[1] TWM, second session, held via electronic means, from April 8 to 11, 2024. See document TWM/2/21 “Report”, paragraphs 57 to 61.

[2] TWV, fifty-eighth session, held via electronic means, from April 22 to 25, 2024. See document TWV//58/11 “Report”, paragraphs 54 to 58.

CONFIDENTIALITY AND OWNERSHIP OF MOLECULAR INFORMATION

Background

12. The TC, at its fifty-eighth session[3], noted discussions held at the TWPs, at their sessions in 2022, on "Confidentiality and Ownership of Molecular Information". The TC noted the concerns expressed by breeders' organizations at the TWM 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 agreed to invite members and observers to report on existing policies on confidentiality of molecular information at the TWPs, at their sessions in 2023 (see document TC/58/31 "Report", paragraphs 48 to 50).

13. The TC, at its fifty-ninth session[4], noted the policies reported on and discussions on confidentiality of molecular information at the TWP sessions in 2023. The TC agreed to repeat the invitation for members and observers to report on existing policies on confidentiality of molecular information at the TWPs, at their sessions in 2024.

14. Further background to this matter is provided in document SESSIONS/2023/5 "Molecular Techniques".

Developments at the Technical Working Parties at their sessions in 2024

15. At their sessions in 2024, the TWPs were invited to make presentations and report on examples of policies on confidentiality and access to molecular data. No reports on existing policies on confidentiality were reported at the TWA, TWF, TWO and TWV.

Technical Working Party on Testing Methods and Techniques (TWM)

16. The TWM[5] received a presentation on "Confidentiality of Molecular Information" from Mr. Marcel Bruins, CropLife International, on behalf of the African Seed Trade Association (AFSTA), the Asia and Pacific Seed Association (APSA), the International Community of Breeders of Asexually Reproduced Horticultural Plants (CIOPORA), CropLife International, Euroseeds, the International Seed Federation (ISF) and the Seed Association of the Americas (SAA) ("breeders' organizations"). A copy of the presentation is provided in document TWM/2/7.

17. The TWM noted the request from breeders' organizations for the development of guidance in UPOV on confidentiality of molecular data and the offer to propose a draft model agreement template, to be presented at its third session.

Examples of policies on confidentiality and access to molecular information data

18. The TWM noted that the European Union was expected to adopt a policy on access to plant variety samples, including DNA samples, which would be reported at the TWPs in 2024.

19. The TWM agreed to invite UPOV members to report on existing policies on confidentiality of molecular information at its third session.

20. The TC is invited to note the request from breeders' organizations for the development of guidance in UPOV on confidentiality of molecular data and the offer to propose a draft model agreement template, to be presented at the third session of the TWM.

[3] TC, fifty-eighth session, held in Geneva, on October 24 and 25, 2022.

[4] TC, fifty-ninth session, held in Geneva, on October 23 and 24, 2023.

[5] TWM, second session, held via electronic means, from April 8 to 11, 2024. See document TWM/2/21 "Report", paragraphs 57 to 61.

MATTERS FOR INFORMATION

Developments at the second session of the Technical Working Party on Testing Methods and Techniques (TWM)

21. The TWM held its second session via virtual means from April 8 to 11, 2024. The following sections report developments on molecular techniques.

Developments in molecular techniques and bioinformatics

Latest developments in molecular techniques and bioinformatics

WIPO Standard ST.26 - WIPO Sequence

22. The TWM received a presentation from Ms. Emma Francis, World Intellectual Property Organization (WIPO) on "WIPO Standard ST.26 - WIPO Sequence", a copy of which is provided in document TWM/2/15.

23. The TWM noted that search algorithms could be developed for databases containing nucleotide or amino acid information using the WIPO Standard ST.26 data format, including plant variety data.

Cooperation between international organizations

OECD

24. 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/2/19.

25. The TWM noted the use of molecular techniques in the OECD Seed Schemes as a supplementary procedure for variety identification in field trials.

26. The TWM noted that OECD was considering the assessment of characteristics using image analysis and that the use of artificial intelligence algorithms was anticipated to be considered in the future.

ISTA

27. The TWM received a presentation from Ms. Ana Laura Vicario, International Seed Testing Association (ISTA), on "ISTA report on the use of molecular techniques", a copy of which is provided in document TWM/2/18.

28. The TWM noted the invitation for interested experts to join the activities of the ISTA Variety Committee.

29. The TWM thanked OECD and ISTA for reporting on developments on the use of molecular techniques in their respective organizations.

30. The TWM noted the invitation from UPOV for the joint organization of an OECD, ISTA and UPOV workshop in the future to discuss the use of molecular techniques in each organization and explore further collaboration in this area.

Report on the work on molecular techniques in relation to DUS examination

Reference collection management using molecular markers: a new approach based on genomic prediction

31. The TWM considered document TWM/2/4 and received a presentation from Mr. Adrian Roberts (United Kingdom) on "Genomic prediction for reference collection management", a copy of which is reproduced in document TWM/2/4 Add.

32. The TWM noted that the genomic prediction method was aimed at establishing links between molecular markers and phenotypic expression of characteristics in ryegrass varieties, and that it might potentially assist in the management of variety collections.

33. The TWM noted that the genomic prediction method had been developed using data from a single trial location and would be further evaluated on other crops where data was available from different locations.

Uniformity assessment using molecular markers

34. The TWM considered document TWM/2/5 and received a presentation from Mr. Adrian Roberts (United Kingdom) on “Uniformity assessment using molecular markers”, a copy of which is reproduced in document TWM/2/5 Add.

35. The TWM noted that the research had been conducted assessing the genetic variability of a cross-pollinated crop (ryegrass) with measured characteristics and not tested on pseudo-qualitative characteristics.

36. The TWM noted that next steps of the research could investigate measurement error associated with the sequencing methodology through independent runs with the same pooled sample.

Molecular approaches to support DUS testing

37. The TWM considered document TWM/2/6 and received a presentation from Ms. Vanessa McMillan (United Kingdom) on “Molecular approaches to support DUS testing”, a copy of which is reproduced in document TWM/2/6 Add.

38. The TWM noted that up to 75% of marker-trait correlation had been achieved in barley varieties, although not in relation to DUS characteristics. The TWM noted the intention to publish the molecular markers identified in the project, which could also be used for authenticating new seedstock of varieties. The TWM agreed to invite the expert from the United Kingdom to report on progress at its third session.

CPVO R&D activities

39. The TWM received a presentation from Ms. Cécile Collonnier (European Union) on “CPVO R&D activities”, a copy of which is provided in document TWM/2/12.

40. The TWM noted the contributions of the various projects presented, in particular the INVITE project, which would end in 2024.

Maize6H-60K: A genome-wide single nucleotide polymorphism array and its application

41. The TWM received a presentation from Ms. Hongli Tian (China) on “Maize6H-60K: A genome-wide single nucleotide polymorphism array and its application”, a copy of which is provided in document TWM/2/16.

42. The TWM noted that 21% of SNPs in the array were located in coding regions of the genome although their link with the expression of characteristics had not yet been identified.

Guidelines for the validation of a new characteristic-specific molecular marker protocol for DUS studies as an alternative method for observation

43. The TWM received a presentation from Ms. Amandine LeVan (France) on “Guidelines for the validation of a new characteristic-specific molecular marker protocol for DUS studies as an alternative method for observation”, a copy of which is provided in document TWM/2/17.

44. The TWM noted that the proposal would be considered by the TWV and reported to the TC, at their sessions in 2024.

The use of molecular techniques in variety identification

Use of Artificial Intelligence-based Markers for Variety Traceability

45. The TWM received a presentation from Ms. Ana Laura Vicario (Argentina) on “Use of Artificial Intelligence-based Markers for Variety Traceability”, a copy which is provided in document TWM/2/9.

46. The TWM noted that the technology was used in routine procedures for market control and traceability of barley and wheat varieties in Argentina. The TWM noted that the technology was being developed for soybean varieties.

47. The TWM noted that the algorithm used established unique patterns for each variety based on seed morphology. The TWM noted that the thresholds for decision making and accepted error could be adjusted to enable the analysis of variety purity.

LociScan, a tool for screening genetic marker combinations for plant variety discrimination

48. The TWM received a presentation from Mr. Yang Yang (China) on “LociScan, a tool for screening genetic marker combinations for plant variety discrimination”, a copy of which is provided in document TWM/2/14.

49. The TWM noted that the software tool LociScan identified marker set combinations to optimize the number of markers required to discriminate varieties. The TWM noted that the time of analysis required by the tool would be influenced by the number of samples processed and not by the number of markers used.

50. The TWM noted that the software tool LociScan was available for testing and agreed to invite interested experts to test the tool and report results to the expert from China.

51. The TC and CAJ are invited to note the matters for information provided in this document.

[Annex follows]

SESSIONS/2024/6

ANNEX

EXTRACT FROM DOCUMENT TWV/58/9

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

ASSOCIATED DOCUMENTS

1. Please note that the parts highlighted indicate text quoted from the documents below:

- 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/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

2. 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 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.

3. 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

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

Requirement

5. 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. It can also suggest that the non-correlation is caused not by the marker but by external factors in the phenotypical observations (*e.g.* biotest for a disease resistance).

How to evaluate it

6. 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*."

7. Varieties should represent the different states of expression (if known varieties with heterozygous and homozygous state), coming from different seed companies, with different genetic background of the characteristic and different types. Well phenotypically characterized varieties for the trait of interest should be used when available.

8. Number of plants per variety: At least one plant per variety if there is available well phenotypically characterized varieties. If not, the number of plants must be the same as for the morphological observation described in the UPOV guideline.

9. The specificity can be assessed within one laboratory.

Sensitivity and limit of detection

Definition

10. The limit of detection is defined as the minimal quantity of the target that can be reliably detected.

11. 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

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

How to evaluate it?

13. 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)

14. "*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.*"

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

Requirement

16. Ideally 100%, a performance ≥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?

17. The repeatability can be evaluated within one laboratory.

18. 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)

19. "*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.

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

Requirement

21. Ideally 100%, a performance ≥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?

22. 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.

23. The ring-test should involve at least, three different laboratories including at least two different examination offices (*e.g.* in the INVITE project 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, the reproducibility can be determined within one laboratory.

24. All laboratories must follow the protocol to be validated. In the protocol compulsory and optional parts can be defined by the validation team. For example, see the protocol CPVO/TP-044/4-Rev. where compulsory and optional steps were defined.

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

26. 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

27. "*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

28. 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?

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

IV. VALIDATION REPORT

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

31. 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

32. 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 shared outside of the examination offices.

V. STANDARD PROTOCOL FOR CHARACTERISTIC-SPECIFIC MOLECULAR MARKER PROTOCOL

33. 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 (see document TWV/54/7 “Use of molecular techniques in DUS examination”. Modifications are highlighted in grey)

Chapter	Elements in a Standard characteristic-specific molecular marker protocol	Example	Essential information for harmoni-zation	Remark
1	characteristic	Resistance to Tomato mosaic virus (ToMV) *See TG/44/11/rev3 – Ad 51: ii DNA marker test*	YES	
2	Genes and alleles	*See TG/44/11/rev3 – Ad 51: ii DNA marker test add 2*	YES	Need to avoid dominant marker or presence/absence marker otherwise the robustness should be assessed
2.1	Targeted gene(s)	Resistance Gene Tm2 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 state 1	Tm2 and Tm2^2 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	tm2 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)	*See TG/44/11/rev3 – Ad 51: ii DNA marker test add 3, 3.1 and 3.2*	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’

Chapter	Elements in a Standard characteristic-specific molecular marker protocol	Example	Essential information for harmoni-zation	Remark
4	Format of the test			
4.1	Number of plants per genotype	>=20	YES	A minimal number of individual plants required (see 5.2.1a) 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 (TGP 15)
4.2	Control varieties	*See TG/44/11/rev3 – Ad 51: ii DNA marker test add 4.2*	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 (susceptible) and heterozygous (both alleles are present in a diploid) corresponding to either resistant, susceptible or intermediate state of expression (depending on gene function; dominant - recessive). DNA controls can be directly used.
4.3	Process controls	*e.g. buffer used for extraction; a marker targeting the cytochrome oxidase gene as an internal amplification marker*	YES	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	*e.g.* Sampling of seedlings 4 days old followed by DNA extraction using CTAB method	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 institute that developed the marker
6	~~Performance or~~ Technique of the method	*e.g.* conventional PCR, TETRA-ARMS, qPCR, KASP, amplicon sequencing *See TG/44/11/rev3 – Ad 51: ii DNA marker test add 6*	YES	.
6.1	Particular conditions	*e.g.* PCR protocol describing primer, enzyme, dNTP concentrations, PCR cycle scheme	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 institute that developed the marker
6.2	Particular hardware or infrastructure	*e.g.* machines, commercial kits, manufactures of components, lot numbers of chemicals	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 institute that developed the marker
7	Observations	*e.g.* Bands on agarose gel (conventional PCR), Ct values (qPCR) Variant call based on sequencing reads	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 institute that developed the marker

Chapter	Elements in a Standard characteristic-specific molecular marker protocol	Example	Essential information for harmoni-zation	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: ii DNA marker test add 8*	YES	Relation between alleles and expressions (with its notes)
9	Validation of the method,	This protocol was validated by a ring-test with different laboratories (*e.g.* Interlaboratory Comparative Test Report, INVITE 2024).	YES	
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

34. 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 double testing at least during the first year with observation method.

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

[End of Annex and of document]