UPOV
International Union for the Protection of New Varieties of Plants

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Technical Working Party for Vegetables
Sixtieth Session
Pacific Grove, United States of America, May 18 to 21, 2026

Technical Working Party on Testing Methods and Techniques
Fourth Session
Cambridge, United Kingdom, June 2 to 5, 2026

Technical Working Party for Agricultural Crops
Fifty-Fifth Session
Seoul, Republic of Korea, June 15 to 18, 2026

Technical Working Party for Ornamental Plants and Forest Trees
Fifty-Eighth Session
Virtual meeting, July 6 to 9, 2026

Technical Working Party for Fruit Crops
Fifty-Seventh Session
Leipzig, Germany, September 7 to 10, 2026

TWP/10/1

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DEVELOPMENT OF GUIDANCE AND INFORMATION MATERIALS

Document prepared by the Office of the Union

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EXECUTIVE SUMMARY

1. The purpose of this document is to provide an overview of the development of UPOV guidance and information materials.

Matters for consideration:

2. The Technical Working Parties (TWPs) are invited to consider proposals to revise document TGP/7 "Development of Test Guidelines" in relation to:

- universal standard wording in Test Guidelines for "number of growing cycles";
- additional standard wording on "number of growing cycles";
- additional standard wording on "explanation of the growing cycles".

3. The Technical Working Party on Testing Methods and Techniques (TWM) is invited to review the software "KORA" and make a recommendation for consideration by the Technical Committee on whether to include the proposed software in document UPOV/INF/16 "Exchangeable Software".

Matters for information:

4. The TWPs are invited to note information on the revision of the following documents to be put forward for adoption by the Council in 2026:

- TGP/5 "Experience and Cooperation in DUS Testing", Section 11 "Examples of Policies and Contracts for Material Submitted by the Breeder";
- TGP/15 "Guidance on the Use of Biochemical and Molecular Markers in the Examination of Distinctness, Uniformity and Stability (DUS)" (Revision); New section: "Guidelines for the validation of a new characteristic-specific molecular marker protocol as an alternative method for observation";
- UPOV/INF/16 "Exchangeable Software"
- UPOV/INF/22 "Software and Equipment Used by Members of the Union"

5. The TWPs are invited to note information on the revision of the following documents adopted by the Council in 2025:

- TGP/5: Experience and Cooperation in DUS Testing: Section 6 “UPOV Report on Technical Examination and UPOV Variety Description”
- TGP/7: Development of Test Guidelines, Guidance Note 28 “Example Varieties”: Situations where illustrations could complement or replace example varieties

6. The structure of this document is as follows:

7. The following abbreviations are used in this document:

CAJ:	Administrative and Legal Committee
TC:	Technical Committee
TWA:	Technical Working Party for Agricultural Crops
TWF:	Technical Working Party for Fruit Crops
TWM:	Technical Working Party on Testing Methods and Techniques
TWO:	Technical Working Party for Ornamental Plants and Forest Trees
TWV:	Technical Working Party for Vegetables
TWPs:	Technical Working Parties

MATTERS FOR CONSIDERATION BY THE TECHNICAL WORKING PARTIES (TWPS)

Revision of document TGP/7 “Development of Test Guidelines”: Number of growing cycles and concluding examination

Background

8. UPOV Test Guidelines are prepared or revised according to the structure and universal standard wording that is appropriate for all Test Guidelines, as provided in document TGP/7.

9. The complete background to this matter is provided in Annex I to this document.

Developments in 2025

10. At its session in 2025, the TWF[1] agreed to propose amending the Test Guidelines structure and universal standard wording, to replace the term “normally” by “generally” in the Additional Standard Wording (ASW) 2 on “Number of growing cycles”; and to present consecutively ASW 2 and the sentence “The testing of a variety may be concluded when the competent authority can determine with certainty the outcome of the test.

11. In October 2025, the TC[2] considered a proposal to amend document TGP/7 and agreed to invite the TWPs, at their sessions in 2026, to consider the proposed amendments in relation to the number of growing cycles and concluding examination, as provided in this document.

Proposals

12. The following section presents proposals for the revision of document TGP/7 “Development of Test Guidelines” in relation to the universal standard wording in Test Guidelines for “number of growing cycles” and when the testing of a variety may be concluded. The proposals include the revision of additional standard wording (ASW) on “number of growing cycles” (ASW 2) and “Explanation of the growing cycles” (ASW 3).

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1 TWF, fifty-sixth session, held at Bursa, Türkiye, from June 23 to 26, 2025. See document TWF/56/3 “Report”, paragraphs 10 to 19

2 TC, sixty-first session, held in Geneva on October 20 and 21, 2025. See document TC/61/8 “Report”, paragraphs 23 to 25.

Test Guidelines Structure and Universal Standard Wording

13. The TWPs are invited to consider the proposal to amend the structure and universal standard wording of Test Guidelines, to read as follows:

"3. Method of Examination

"3.1 Number of Growing Cycles

"The ~~minimum~~ duration of tests should ~~normally~~ generally be:

"{ **ASW 2** (Chapter 3.1(.1)) – number of growing cycles }

"The testing of a variety may be concluded when the competent authority can determine with certainty the outcome of the test.

"{ GN 8 (Chapter 3.1.2) – explanation of the growing cycle }
"{ **ASW 3** (Chapter 3.1.2) – explanation of the growing cycle }

~~The testing of a variety may be concluded when the competent authority can determine with certainty the outcome of the test.~~"

ASW 2 "Number of growing cycles"

14. The TWPs are invited to consider the proposal to amend the additional standard wording of Test Guidelines, to read as follows:

"(a) Single growing cycle:

"The ~~minimum~~ duration of tests should ~~normally~~ generally be a single growing cycle. The testing of a variety may be concluded when the competent authority can determine with certainty the outcome of the test"

"(b) Two independent growing cycles:

"The ~~minimum~~ duration of tests should ~~normally~~ generally be two independent growing cycles. The testing of a variety may be concluded when the competent authority can determine with certainty the outcome of the test"

ASW 3 (d) "Explanation of the Growing Cycle: Fruit species"

14. The TWPs are invited to consider the proposal to amend the additional standard wording on explanation of growing cycle for fruit species to read as follows (ASW 3):

"(d) Fruit species

"In the case of Test Guidelines covering fruit species, the following sentence may be added in Chapter 3.1:

"In particular, it is essential that the [trees] / [plants] produce a ~~satisfactory crop~~ sufficient quantity and quality of fruit for testing purposes and are representative of the variety in any ~~in each of the two~~ growing cycle~~s~~."

15. The TWPs are invited to consider the proposed amendments to document TGP/7 "Development of Test Guidelines", to amend the universal standard wording for "number of growing cycles" and to when the testing of a variety may be concluded, as set out in paragraphs 12 to 14 of this document.

MATTERS FOR CONSIDERATION BY THE TECHNICAL WORKING PARTY ON TESTING METHODS AND TECHNIQUES (TWM)

UPOV/INF/16 "Exchangeable Software" (Revision)

Software proposed for inclusion in document UPOV/INF/16

15. On January 19, 2026 the Office of the Union issued Circular E-26/004 to all UPOV bodies inviting them to provide information on software that had been developed by PVP authorities they would wish to make available to other UPOV members in document UPOV/INF/16.

16. In response to Circular E-26/004, the Office of the Union received a proposal from Italy to include in document UPOV/INF/16 the software "KORA", with the following information:

Program name	Programming language	Function (brief summary)	Source & contact details
KORA	Python	Computes comparisons of varieties for management of reference collections	Italy Ms. Anna Giulini Mr. Gabriele Mongiano

17. In accordance with the procedure in document UPOV/INF/16, the software "KORA" will be presented for review by the TWM, at its fourth session. The TWM will be invited to make a recommendation for consideration by the TC, at its sixty-second session, on whether to include the proposed software in document UPOV/INF/16.

18. The TWM is invited to review the software "KORA" and make a recommendation for consideration by the TC, at its sixty-second session, on whether to include the proposed software in document UPOV/INF/16.

MATTERS FOR INFORMATION

Document TGP/5 "Experience and Cooperation in DUS Testing"

Section 11 "Examples of Policies and Contracts for Material Submitted by the Breeder" (Revision)

19. The TC[3], in October 2025, noted the "Policy on the status of plant material submitted for DUS testing purposes" reported by the European Union at the TWA, as provided in the Annex II to this document (see: https://cpvo.europa.eu/en/cpvo-policy-status-plant-material-used-dus-testing-purposes).

20. The TC noted that examples of policies and contracts for material submitted by the breeder were provided in document TGP/5 "Experience and Cooperation in DUS Testing", Section 11 "Examples of Policies and Contracts for Material Submitted by the Breeder" (Revision). Following the TC session, the European Union requested the UPOV Office to update the policy currently provided in document TGP/5, Section 11, which should be replaced by the current policy provided in the Annex to this document.

21. Subject to agreement by the CAJ, at its eighty-third session, an agreed revision of document TGP/5, Section 11 would be put forward for adoption by the Council, at its sixtieth ordinary session, to replace the policy of the Community Plant Variety Office (CPVO) of the European Community with regard to the status of plant material submitted for DUS testing purposes.

[3] TC, sixty-first session, held in Geneva on October 20 and 21, 2025. See document TC/61/8 "Report", paragraph 60.

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

New section: “Guidelines for the validation of a new characteristic-specific molecular marker protocol as an alternative method for observation”

22. Document TGP/15 provides guidance on the use of biochemical and molecular markers in the examination of Distinctness, Uniformity and Stability (DUS).

23. The TC[4], at its sixty-first session, considered draft guidance on how to validate “characteristic-specific” molecular markers for DUS examination and a standard template to describe their use to assess characteristics in Test Guidelines.

24. The TC considered the methodology proposed and agreed on the importance of harmonized validation for new molecular markers proposed for inclusion in Test Guidelines as alternative methods. The TC agreed that guidance should allow for other methods that achieve the same result, provided they are also validated.

25. The TC discussed the situation described in paragraph 31 of the draft guidance on matters to be considered on the use of molecular markers that might be trade secrets and agreed that these situations should be considered on a case-by-case basis when developing characteristics for Test Guidelines.

26. The TC agreed to propose to the Council adopting the guidance for the validation of characteristic specific molecular markers as alternative methods for the assessment of characteristics in Test Guidelines, as set out in the Annex to document SESSIONS/2025/6, subject to linguistic checking by the Enlarged Editorial Committee (TC-EDC).

27. The TC-EDC, at its meetings held on January 13 and 15, 2026, considered the draft guidance and recommended for adoption by the Council, at its sixtieth session, to be held in Geneva on October 23, 2026, subject to editorial clarifications, as provided in Annex III to this document.

Document UPOV/INF/16 “Exchangeable Software” (Revision)

Adoption of document UPOV/INF/16

28. Based on the recommendations of the Consultative Committee in October 2025, the Council will be invited to amend the “Procedure for inclusion of the software” in document UPOV/INF/16, so that future revisions of that document will not require formal adoption by the Council, as follows:

> “2. Procedure for inclusion of software
>
> “Software proposed for inclusion in document UPOV/INF/16 by members of the Union is, in the first instance, presented for review by the Technical Working Party on Testing Methods and Techniques (TWM). On the basis of such presentations and the experience of members of the Union, the TWM makes a recommendation to the Technical Committee (TC) on whether to include that software in document UPOV/INF/16. In the case of a positive recommendation by the TC ~~and by the Administrative and Legal Committee (CAJ)~~, the software will be listed in a ~~draft~~ a revised version of document UPOV/INF/16~~, to be considered for adoption by the Council. Document UPOV/INF/16 is adopted by the Council~~ and published on the UPOV website.”

Document UPOV/INF/22 “Software and Equipment Used by Members of the Union” (Revision)

Adoption of document UPOV/INF/22

29. Based on the recommendations of the Consultative Committee in October 2025, the Council will be invited to adopt the following amendment to the “Procedure for inclusion of the software/equipment” in document UPOV/INF/22, so that future revisions of that document will not require formal adoption by the Council:

[4] TC, sixty-first session, held in Geneva, from October 20 to 21, 2025. See document TC/61/8 “Report”, paragraphs 54 to 56

"2. Procedure for inclusion of software/equipment

"2.1 Software/equipment proposed for inclusion in this document by members of the Union is, in the first instance, presented to the Technical Committee (TC).

"2.2 The TC will decide whether to:

(a) propose to include the information in the document;
(b) request further guidance from other relevant bodies (e.g. the Administrative and Legal Committee (CAJ) and the Technical Working Parties (TWPs)); or
(c) propose not to include the information in the document.

"2.3 In the case of a positive recommendation by the TC ~~and, subsequently by the CAJ,~~ the software/equipment will be listed in a revised version ~~draft~~ of the document~~, to be considered for adoption by the Council~~ and published on the UPOV website."

Software proposed for inclusion in document INF/16

30. On January 19, 2026 the Office of the Union issued Circular E-26/004 to all UPOV bodies inviting them to provide information on software and equipment used by members of the Union for PVP purposes.

31. In response to Circular E-26/004, the Office of the Union received proposals for the inclusion of information on software and equipment used by: China, the Netherlands (Kingdom of), Sweden and Ukraine.

32. The proposals to include new software or equipment in document UPOV/INF/22 will be considered by the Technical Committee (TC) at its sixty-second session, to be held on October 19 and October 20, 2026.

Guidance and information materials adopted by the Council in 2025

Document TGP/5: Experience and Cooperation in DUS Testing - Section 6 "UPOV Report on Technical Examination and UPOV Variety Description" (Revision) (document TGP/5, Section 6/5)

33. In October 2025, the Council adopted a revision of document TGP/5 "Experience and Cooperation in DUS Testing", Section 6 "UPOV Report on Technical Examination and UPOV Variety Description", on the basis of document TGP/5, Section 6/5 Draft 2.

34. Document TGP/5, Section 6 provides a standard model to report on the examination of a plant variety for distinctness, uniformity and stability (DUS). The proposed revision was aimed at increasing the takeover of DUS test reports through providing information on similar variety(ies) and the basis to distinguish the candidate variety from these variety(ies).

Document TGP/7: Development of Test Guidelines (Revision) Guidance Note 28 "Example Varieties": Situations where illustrations could complement or replace example varieties

35. In October 2025, Council adopted a revision of document TGP/7 "Development of Test Guidelines", Guidance Note 28 "Example Varieties".

36. Example varieties are used to clarify the states of expression of characteristics in UPOV Test Guidelines. The revision of Guidance Note 28 brings new text to clarify situations where illustrations could complement or replace example varieties to clarify the situations where diagrams and illustrations could be used to replace example varieties to clarify the states of expression of characteristics.

37. The Technical Working Parties are invited to note the developments reported in this document.

[Annex I follows]

ANNEX I

BACKGROUND TO TWF PROPOSAL ON "NUMBER OF GROWING CYCLES AND CONCLUDING EXAMINATION OF FRUIT CROPS"

Developments in 2024

1. At its session in 2024, the TWF[5] received a presentation on "Number of growing cycles and concluding examination of fruit crops" from an expert from the European Union. A copy of the presentation is provided in document TWF/55/4 (see document TWF/55/9 "Report", paragraphs 33 to 37).

2. The TWF noted that the number of growing cycles in Test Guidelines for fruit crops was usually two. The TWF noted that the standard wording for such cases stated that "the minimum duration of tests should normally be two independent growing cycles."

3. The TWF noted that the choice of number of growing cycles for fruit crops was a subject of discussion by the interested experts and the TWF. The TWF noted the experiences reported by Canada and France on assessments conducted after one satisfactory crop of fruits.

4. The TWF considered the standard wording "the testing of a variety may be concluded when the competent authority can determine with certainty the outcome of the test" and whether it could be contradictory to the standard wording that "the minimum duration of tests should normally be two independent growing cycles."

5. The TWF agreed to invite the experts from France with the support of Canada, European Union, France, Germany, New Zealand, Republic of Korea and CIOPORA to develop proposals on the number of growing cycles for fruit crops, such as reducing the duration of tests to one growing cycle for fruit crops and the meaning of "a satisfactory crop of fruit".

Developments in 2025

6. The TWF[6] considered document TWF/56/3, as presented by an expert from Canada (see document TWF/56/7 "Report", paragraphs 10 to 19).

7. The TWF discussed situations when two growing cycles would be required for the expression of characteristics to be sufficiently consistent and clear, according to UPOV guidance, and to generate reliable variety descriptions.

8. The TWF noted the comments from Japan and the Republic of Korea on how UPOV guidance was interpreted in those countries providing flexibility for authorities to decide when two growing cycles would be required, or examination could be concluded when the authority could determine with certainty the outcome of the test.

9. The TWF considered the standard wording for number of growing cycles in Test Guidelines, in particular the sentences on "number of growing cycles" and that "The testing of a variety may be concluded when the competent authority can determine with certainty the outcome of the test." The TWF agreed that the guidance in Test Guidelines could be improved to further clarify that authorities could conclude the examination of fruit crops earlier than two growing cycles, when that was recommended in Test Guidelines.

10. The TWF considered the use of the terms "minimum" and "normally" in relation to the minimum duration of tests and agreed to propose amending document TGP/7, Additional Standard Wording (ASW) 2 to replace the term "normally" by "generally", as follows:

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5 TWF, fifty-fifth session, held by virtual means, from June 3 to 6, 2024.

6 TWF, fifth-sixth session, held in Bursa, Türkiye, from June 23 to 26, 2025.

ASW 2 (Chapter 3.1) – Number of growing cycles

(a) Single growing cycle

"The minimum duration of tests should ~~normally~~ generally be a single growing cycle."

(b) Two independent growing cycles

"The minimum duration of tests should ~~normally~~ generally be two independent growing cycles."

11. The TWF noted that the sequence of standard wording in Test Guidelines presented the explanation on "number of growing cycles" separated from the explanation that "The testing of a variety may be concluded when the competent authority can determine with certainty the outcome of the test". The TWF agreed that the latter sentence was an important explanation of the number of growing cycles and agreed to propose amending the "TG Structure and Universal Standard Wording" to present consecutively both sentences, as follows:

ANNEX 1: TG STRUCTURE AND UNIVERSAL STANDARD WORDING

3. Method of Examination

3.1 Number of Growing Cycles

The minimum duration of tests should ~~normally~~ generally be:

{ **ASW 2** (Chapter 3.1(.1)) – number of growing cycles }

The testing of a variety may be concluded when the competent authority can determine with certainty the outcome of the test.

{ GN 8 (Chapter 3.1.2) – explanation of the growing cycle }
{ **ASW 3** (Chapter 3.1.2) – explanation of the growing cycle }

~~The testing of a variety may be concluded when the competent authority can determine with certainty the outcome of the test.~~

12. The TWF noted the comment from the European Union that the standard sentence on "concluding testing" should not be interpreted as contradictory to the standard wording on number of growing cycles, in particular that the testing of a variety may be concluded earlier.

13. The TWF agreed to propose considering whether the provision on "concluding testing" should be added to the different standard wording options in ASW 2 "Number of growing cycles" to ensure that the basic principles contained in the General Introduction could be used, rather than following the detailed recommendations of the Test Guidelines.

14. The TWF considered a proposal to amend Additional Standard Wording 3 (ASW 3) for fruit species to clarify the notion of "satisfactory crop of fruit", as provided in document TWF/56/3, as follows:

"In particular, it is essential that the [trees] / [plants] produce a ~~satisfactory crop~~ sufficient quantity of fruit for testing purposes and are representative of the variety in any ~~in each~~ ~~of the two~~ growing cycle~~s~~. Testing of a variety should begin in the following growing cycle after trial trees have had at least one crop of fruit."

15. The TWF agreed there was no need to provide guidance to avoid examining plants / trees in juvenile stage, as this was already covered by the word “representative”. The TWF agreed that the term “satisfactory” could be defined in relation to quantity, quality and representativeness of a crop of fruit of the variety. The TWF agreed to propose amending guidance in document TGP/7, ASW 3 (d) “Fruit species” to read as follows:

“ASW 3 (Chapter 3.1.2) – Explanation of the growing cycle

[…]

“(d) Fruit species

“In the case of Test Guidelines covering fruit species, the following sentence may be added in Chapter 3.1:

“In particular, it is essential that the [trees] / [plants] produce a ~~satisfactory crop~~ sufficient quantity and quality of fruit for testing purposes and are representative of the variety in any ~~in each~~ ~~of the two~~ growing cycle~~s~~.”

[Annex II follows]

CPVO
Community Plant Variety Office

CPVO POLICY ON THE STATUS OF PLANT MATERIAL SUBMITTED FOR DUS TESTING PURPOSES

The aim of this document is to make transparent the policy of the CPVO concerning material sent for DUS testing in the framework of Community Plant Variety Right applications. It provides guidance for the implementation of article 88.4 of Council Regulation (EC) 2100/94 as well as article 13.3 of COMMISSION REGULATION (EC) No 874/2009. It will also contribute to a coherent practice by all entrusted Examination Offices in the CPVO. This will permit breeders to make an informed decision before sending material for testing. It is not the competence of the CPVO to decide what examination offices may do in relation to material submitted in the framework of a national PVR application or for national listing purposes. Accordingly, the CPVO cannot assure breeders that the below policy has been applied when the CPVO takes over reports from tests which has been carried out or is in the process of being carried out. The CPVO would nevertheless urge examination offices to follow the same principles when testing varieties for purposes other than in the framework of Community Plant Variety Right applications.

The policy does not apply to any examination offices other than examination offices entrusted by the Administrative Council of the CPVO for the same given species (hereinafter "**Examination Office**"). Accordingly, when the below mentioned policy refers to a transfer of material between two Examination Offices, this relates only to material of a given species that both the sending and receiving Examination Offices are entrusted to test by the Administrative Council of the CPVO.

Reference to "**material**" in this policy also applies to DNA samples taken by an Examination Office from plant material submitted to it for DUS purposes.

1. What should an Examination Office do with material if the application is withdrawn or rejected?

1.1. The Examination Office should either destroy or send back the material to the applicant.

1.2. If the variety is of common knowledge, the Examination Office may keep the material in its reference collection.

2. May an Examination Office send material…

2.1. To another Examination Office?

2.1.1 On request, the Examination Office should send material to another Examination Office.

2.1.2 If the material concerned consists of parent lines or would disclose information on hybrid formulas, the Examination Office should inform the person entitled that the material has been sent to another Examination Office

2.1.3 The Examination Office shall not use the material received from another Examination Office for any other purposes than for DUS tests or R&D projects between Examination Offices only aiming to improve DUS testing.

If the R&D projects aiming to improve DUS testing involve any non-entrusted examination office or any third party, consent of the title holder is required. The provisions on confidentiality and conflicts of interest in the Designation Agreement between the CPVO and the Examination Office shall apply and be reflected in the consortium agreement signed by the partners of the R&D projects.

2.1.4 If the material is used for any other purposes than those mentioned under 2.1.1 - 2.1.3, consent of the title holder is required. In that case also, the provisions on confidentiality and conflicts of interest in the Designation Agreement between the CPVO and the Examination Office shall apply.

2.2 To a non-entrusted examination office or a certification authority or any other entity?

2.2.1 On request, the Examination Office may send material to another non-entrusted examination office only if consent from the person entitled has been obtained and delivered (or referred to) by this other non-entrusted examination office.

2.2.2 The Examination Office may provide material to a certification authority on request only if consent has been obtained from the person entitled and delivered (or referred to) by the certification authority.

2.2.3 Furthermore, the Examination Office may provide material to any other entity only if consent from the person entitled has been obtained and delivered (or referred to) by this other entity.

3. What may the Examination Office do with the material after the variety has been granted a Community PVR?

3.1 If the Examination Office does not keep a living reference collection the material shall be destroyed or sent back to the applicant.

3.2 If the Examination Office keeps a living reference collection including DNA samples the material should be kept by the Examination Office.

3.3 If the material is kept, the Examination Office may, on request, transfer material to another Examination Office or to a non-entrusted examination office on the same conditions as provided for in Section 2 above.

4. After the Community Plant Variety Expires

4.1. Material kept in a reference collection should be kept upon expiry of a Community plant variety right.

[Annex III follows]

ANNEX III

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

Table of Contents:

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 and a follow-up survey.

2. If a different technique is used, the laboratory should 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 should 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 ≥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 ≥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. 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 assessment will be possible with different operators.

23. All laboratories should follow the protocol to be validated. In the protocol standard 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 u sing 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, etc.).

IV. VALIDATION REPORT

29. The validation report and results should 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 a 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 standard and optional 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.

V. STANDARD PROTOCOL FOR CHARACTERISTIC-SPECIFIC MOLECULAR MARKER

32. Standard 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 modify a standard chapter it should validate its method in comparison to the reference method.

Table 1: Standard protocol for characteristic-specific molecular marker as alternative method for observation

Chapter	Elements in a Standard characteristic-specific molecular marker protocol	Essential information for harmoni-zation	Remark
1	Characteristic	YES	Name of the characteristic.
2	Genes and alleles	YES	Need to avoid dominant marker or presence/absence marker otherwise the robustness should be assessed.
2.1	Targeted gene(s)	YES	a) file(s) containing the DNA sequence information (order of nucleotides). b) reference to DNA information in public databases (like gene bank). c) reference to 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.

Chapter	Elements in a Standard characteristic-specific molecular marker protocol	Essential information for harmoni-zation	Remark
2.2	Allele corresponding to expression state 1	YES	a) file(s) containing the DNA sequence information (order of nucleotides). b) reference to DNA information in public databases (like gene bank). c) reference to 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	YES	a) file(s) containing the DNA sequence information (order of nucleotides). b) reference to DNA information in public databases (like gene bank). c) reference to 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)	YES	Primer and probe sequences, reference to accessions and sequences in public databases (gene bank 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		
4.1	Number of plants per genotype	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	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 resistance, susceptibility or intermediate resistance of the variety (depending on gene function; dominant - recessive). DNA controls can be directly used.

Chapter	Elements in a Standard characteristic-specific molecular marker protocol	Essential information for harmoni-zation	Remark
4.3	Process controls	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 (a marker targeting the cytochrome oxidase gene as an internal amplification marker). d) Buffer used for extraction.
5	Preparations	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 organization that developed the marker. Mostly sampling of seedlings 4 days old followed by DNA extraction using CTAB method.
6	Technique of the method	YES	Conventional PCR, TETRA-ARMS, qPCR, KASP, amplicon sequencing.
6.1	Particular conditions	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. PCR protocol describing primer, enzyme, dNTP concentrations, PCR cycle scheme.
6.2	Particular hardware or infrastructure	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. Machines, commercial kits, manufactures of components, lot numbers of chemicals.
7	Observations	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. Bands on agarose gel (conventional PCR), Ct values (qPCR) Variant call based on sequencing reads.
7.1	Validity of the results	YES	Depending on the method used. 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).
8	Interpretation of the test results	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.

Chapter	Elements in a Standard characteristic-specific molecular marker protocol	Essential information for harmoni-zation	Remark
9	Validation of the protocol	YES	To provide information on the validation of the protocol or to refer to contact the examination office for further information.
9.1	Contact Examination Office	YES	Contact of the institute that developed the protocol, name of the service.

33 An example of 'characteristic-specific molecular marker as alternative method for observation' is provided in the Test Guidelines for Tomato (TG/44/12), under characteristic 'Resistance to Tomato Mosaic Virus' (ToMV) (see Ad. 59(ii), 'DNA marker test'). Although compliant with the present guidance, it was adopted prior to the standard presentation format outlined in Table 1 and is therefore presented in a different format. The report of the comparative test conducted by three laboratories is available upon request to info@naktuinbouw.nl

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 parallel testing (marker test and bioassay) 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 should be performed.

VII. LITERATURE

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