

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

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REVISION OF DOCUMENT TGP/15 “GUIDANCE ON THE USE OF BIOCHEMICAL AND MOLECULAR MARKERS IN THE EXAMINATION OF DISTINCTNESS, UNIFORMITY AND STABILITY (DUS)”

Document prepared by the Office of the Union

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EXECUTIVE SUMMARY

1. The purpose of this document is to report developments concerning document TGP/15 “Guidance on the Use of Biochemical and Molecular Markers in the Examination of Distinctness, Uniformity and Stability (DUS)” and to present a proposal on the revision of document TGP/15 for inclusion of a new example for Model (1) “Characteristic-specific molecular marker”.

2. The BMT is invited to consider the proposal for a new example to be added to document TGP/15 to illustrate a situation where the characteristic-specific marker does not provide complete information on the state of expression of a characteristic, as set out in the Annex II to this document, including:

(a) whether the request by the TWV is covered in the draft guidance by the following sentence: “6. If a variety is claimed to be resistant to ToMV Strain 0 and the DNA marker test result is tm2/tm2 (homozygous susceptible) a bioassay needs to be performed to determine whether the variety is resistant on the basis of another gene, such as Tm1”;

(b) whether it should be clarified whether the sources of resistance to ToMV Strain 0 were genes Tm1/tm1 and Tm2/Tm2²/tm2 or whether there were other known sources of resistance;

(c) whether it should be clarified whether different markers were linked to the alleles Tm2 and Tm2². In case both alleles were linked to the same marker, the two columns for the resistant alleles should be combined in Table 1 “Schematic overview of resistance to Tomato mosaic virus and resistance alleles.”; and

(d) whether the guidance should clarify that, according to the procedure, varieties claimed as susceptible would also be included in the bioassay.

3. The structure of this document is as follows:

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4. The following abbreviations are used in this document:

BMT:	Working Group on Biochemical and Molecular Techniques, and DNA-Profiling in Particular
CAJ:	Administrative and Legal Committee
TC:	Technical Committee
TWA:	Technical Working Party for Agricultural Crops
TWC:	Technical Working Party on Automation and Computer Programs
TWF:	Technical Working Party for Fruit Crops
TWO:	Technical Working Party for Ornamental Plants and Forest Trees
TWPs:	Technical Working Parties
TWV:	Technical Working Party for Vegetables

REVISION OF THE MODEL "COMBINING PHENOTYPIC AND MOLECULAR DISTANCES IN THE MANAGEMENT OF VARIETY COLLECTIONS"

Background

5. The background to this matter is provided in document BMT/17/7 "Revision of document TGP/15 'Guidance on the Use of Biochemical and Molecular Markers in the Examination of Distinctness, Uniformity and Stability (DUS)'", paragraphs 9 to 18.

6. The Working Group on Biochemical and Molecular Techniques, and DNA-Profiling in Particular (BMT), at its seventeenth session, held in Montevideo, Uruguay, from September 10 to 13, 2018, considered documents BMT/17/7 and TGP/15/2 Draft 1. Document BMT/17/7 contained a revised proposal from France for the revision of document TGP/15, Section 2.2, in response to the comments made by the Technical Working Party for Agricultural Crops (TWA), at its forty-seventh session.

7. The BMT considered the revision of the example of parent lines in maize prepared by the experts from France. The BMT noted that the establishment of an additional threshold for genetic distance below GAIA distance 2 had not been implemented in France at that time. The BMT noted that the nature of document TGP/15 was to present examples of the use of molecular markers in DUS examination among UPOV members. The BMT agreed to recommend that the example in document TGP/15, Section 2.2, be revised at a later stage once the additional threshold level had been implemented in France (see document BMT/17/25 "Report", paragraph 58).

Developments at the fifty-fourth session of the Technical Committee

8. The Technical Committee (TC), at its fifty-fourth session, held in Geneva, from October 29 to 30, 2018, agreed with the BMT that the Model "Combining Phenotypic and Molecular Distances in the Management of Variety Collections" of document TGP/15, Section 2.2, should be revised at a later stage once an additional threshold level has been implemented in France (see document TC/54/31 "Report", paragraph 289).

PROPOSAL FOR INCLUSION OF A NEW MODEL "GENETIC SELECTION OF SIMILAR VARIETIES FOR THE FIRST GROWING CYCLE"

Background

9. The background to this matter is provided in document BMT/17/7 "Revision of document TGP/15 'Guidance on the Use of Biochemical and Molecular Markers in the Examination of Distinctness, Uniformity and Stability (DUS)'", paragraphs 20 to 23.

10. The BMT, at its seventeenth session, considered documents BMT/17/7 and TGP/15/2 Draft 1.

11. The BMT considered the new application model "Genetic Selection of Similar Varieties for the First Growing Cycle" and agreed that it should be proposed for inclusion in document TGP/15 on the basis of a simplified version of draft text presented in document TGP/15/2 draft 1. The BMT agreed that the proposal to be put forward for approval by the TC should contain the description of the method without comparison to other approaches. The BMT also agreed to invite the Netherlands to review whether the schematic explaining the process was necessary and/or might be simplified (see document BMT/17/25 "Report", paragraph 59).

Developments at the fifty-fourth session of the Technical Committee

12. The TC, at its fifty-fourth session, agreed with the inclusion of a new model “Genetic selection of similar varieties for the first growing cycle: example French Bean” in document TGP/15 on the basis of the proposal by the Netherlands revised by the Enlarged Editorial Committee (TC-EDC), as presented in the Annex I to this document (see document TC/54/31, paragraph 291).

13. A draft of document TGP/15/2 “Guidance on the Use of Biochemical and Molecular Markers in the Examination of Distinctness, Uniformity and Stability (DUS)” incorporating the new model “Genetic selection of similar varieties for the first growing cycle: example French Bean” will be presented to the seventy-sixth session of the Administrative and Legal Committee (CAJ), to be held on October 30, 2019.

14. Subject to agreement by the CAJ at its seventy-sixth session, a draft of document TGP/15/2 will be presented for adoption by the Council, at its fifty-third ordinary session, to be held in Geneva on November 1, 2019.

CHARACTERISTIC-SPECIFIC MARKER WITH INCOMPLETE INFORMATION ON STATE OF EXPRESSION

Background

15. The BMT, at its seventeenth session, considered document BMT/17/21 “Do resistance markers for tomato fulfil the requirements of TGP/15?” and received a presentation by Ms. Amanda van Dijk-Veldhuizen (Netherlands), a copy of which was provided as document BMT/17/21 Add. (see document BMT/17/25 “Report”, paragraphs 10 to 12).

16. The BMT agreed that the method presented in document BMT/17/21 was consistent with the model “Characteristic-Specific Molecular Markers” in document TGP/15. The BMT agreed to propose that a new example be added to document TGP/15, on the basis of the example provided by the Netherlands, to illustrate a situation where the characteristic-specific marker did not provide complete information on the state of expression of a characteristic.

17. The BMT agreed to propose that paragraph 3.1.4 (reproduced below) from document UPOV/INF/18/1 be introduced in document TGP/15 to clarify that it was the responsibility of the authority to decide on the reliability of the link between the gene and the expression of the characteristic.

“3.1.4 In considering the model and example, as presented in Annex 1 of this document [Characteristic-specific molecular markers], the TC emphasized the importance of meeting the assumptions. In that regard, it clarified that it is a matter for the relevant authority to consider if the assumptions are met (see document TC/45/16 “Report”, paragraph 152).”

18. When considering whether to include the method in the Test Guidelines, the BMT further proposed that TGP/15 include an explanation that it would be the responsibility of the respective Technical Working Party (TWP) and the TC to assess whether the reliability of the link between the gene and the expression of the characteristic was satisfied.

Developments at the fifty-fourth session of the Technical Committee

19. The TC, at its fifty-fourth session, agreed that the following text from document UPOV/INF/18/1 should be introduced in document TGP/15 to clarify that it was the responsibility of the authority to decide on the reliability of the link between the gene and the expression of the characteristic (see document TC/54/31 “Report”, paragraphs 272 to 275):

“3.1.4 In considering the model and example, as presented in Annex 1 of this document [Characteristic-specific molecular markers], the TC emphasized the importance of meeting the assumptions. In that regard, it clarified that it is a matter for the relevant authority to consider if the assumptions are met (see document TC/45/16 “Report”, paragraph 152).”

20. The TC considered the proposal by the BMT and agreed to include an explanation in document TGP/15 that it would be the responsibility of the respective TWP and the TC to assess whether the reliability of the link between the gene and the expression of the characteristic was satisfied in order to include a method in the Test Guidelines.

21. The TC noted that the BMT, at its seventeenth session, had considered document BMT/17/21 "Do resistance markers for tomato fulfil the requirements of TGP/15" and received a presentation by Ms. Amanda van Dijk-Veldhuizen (Netherlands), a copy of which was provided as document BMT/17/21 Add..

22. The TC agreed with the proposal by the BMT that a new example be added to document TGP/15 to illustrate a situation where the characteristic-specific marker did not provide complete information on the state of expression of a characteristic, on the basis of the proposal by the Netherlands presented in document BMT/17/21. The TC agreed to invite the experts from the Netherlands to prepare a proposal to be presented to the TWPs and BMT and agreed that the resultant proposal should be presented to the TC, at its fifty-fifth session.

Developments at the Technical Working Parties

23. The TWPs, at their sessions in 2019, considered document TWP/3/12 "Characteristic-specific marker with incomplete information on state of expression" (see documents TWO/51/12 "Report", paragraphs 33 to 35, TWV/53/14 "Report", paragraphs 28 to 30, TWF/50/13 "Report", paragraphs 28 and 29, and TWA/48/9 "Report", paragraphs 32 to 35).

24. The TWO, TWV and TWF agreed with the proposed example to be added to document TGP/15 to illustrate a situation where the characteristic-specific marker does not provide complete information on the state of expression of a characteristic, as set out in the Annex II to this document.

25. The TWO noted that disease resistance characteristics were not commonly used in ornamental plants.

26. The TWV agreed that a new sentence should be added to TGP/15 in the case where a variety was claimed by the applicant to be resistant in the TQ but the marker test is negative, a bio-assay should be conducted/performed and be conclusive.

27. The TWA agreed that it should be clarified whether the sources of resistance to ToMV Strain 0 were genes Tm1/tm1 and Tm2/Tm2²/tm2 or whether there were other known sources of resistance.

28. The TWA agreed that it should be clarified whether different markers were linked to the alleles Tm2 and Tm2². In case both alleles were linked to the same marker, the two columns for the resistant alleles should be combined in Table 1 "Schematic overview of resistance to Tomato mosaic virus and resistance alleles."

29. The TWA agreed that the guidance should clarify that, according to the procedure, varieties claimed as susceptible would also be included in the bioassay.

30. Comments by the TWC, at its thirty-seventh session, to be held in Hangzhou, China, from October 14 to 16, 2019, and the BMT, at its eighteenth session, will be reported to the TC, at its fifty-fifth session.

Matters for consideration by the BMT

31. Annex II to this document presents a proposal prepared by experts from the Netherlands for a new example to be added to document TGP/15, Model "Characteristic-specific molecular markers", to illustrate a situation where the characteristic-specific marker does not provide complete information on the state of expression of a characteristic.

32. The BMT may wish to consider whether the request by the TWV in paragraph 28 is covered in the draft guidance by the following sentence:

"6. If a variety is claimed to be resistant to ToMV Strain 0 and the DNA marker test result is tm2/tm2 (homozygous susceptible) a bioassay needs to be performed to determine whether the variety is resistant on the basis of another gene, such as Tm1."

33. The BMT may wish to consider the following proposals by the TWA, as set out in paragraphs 29 to 31 of this document:

(a) that it should be clarified whether the sources of resistance to ToMV Strain 0 were genes Tm1/tm1 and Tm2/Tm2²/tm2 or whether there were other known sources of resistance;

(b) that it should be clarified whether different markers were linked to the alleles Tm2 and Tm2². In case both alleles were linked to the same marker, the two columns for the resistant alleles should be combined in Table 1 "Schematic overview of resistance to Tomato mosaic virus and resistance alleles."; and

(c) that the guidance should clarify that, according to the procedure, varieties claimed as susceptible would also be included in the bioassay.

34. The BMT is invited to consider the proposal for a new example to be added to document TGP/15 to illustrate a situation where the characteristic-specific marker does not provide complete information on the state of expression of a characteristic, as set out in the Annex II to this document, including:

(a) whether the request by the TWV is covered in the draft guidance by the following sentence: "6. If a variety is claimed to be resistant to ToMV Strain 0 and the DNA marker test result is tm2/tm2 (homozygous susceptible) a bioassay needs to be performed to determine whether the variety is resistant on the basis of another gene, such as Tm1";

(b) whether it should be clarified whether the sources of resistance to ToMV Strain 0 were genes Tm1/tm1 and Tm2/Tm2²/tm2 or whether there were other known sources of resistance;

(c) whether it should be clarified whether different markers were linked to the alleles Tm2 and Tm2². In case both alleles were linked to the same marker, the two columns for the resistant alleles should be combined in Table 1 "Schematic overview of resistance to Tomato mosaic virus and resistance alleles."; and

(d) whether the guidance should clarify that, according to the procedure, varieties claimed as susceptible would also be included in the bioassay.

[Annexes follow]

PROPOSAL AGREED BY THE TECHNICAL COMMITTEE (TC) TO AMEND DOCUMENT TGP/15/2 DRAFT 1 "GUIDANCE ON THE USE OF BIOCHEMICAL AND MOLECULAR MARKERS IN THE EXAMINATION OF DISTINCTNESS, UNIFORMITY AND STABILITY (DUS)" NEW MODEL: "GENETIC SELECTION OF SIMILAR VARIETIES FOR THE FIRST GROWING CYCLE"

Genetic Selection of Similar Varieties for the First Growing Cycle

The Technical Committee (TC), at its fifty-fourth session, held in Geneva on October 29 and 30, 2018 agreed to amend document TGP/15/2 Draft 1 "Guidance on the Use of Biochemical and Molecular Markers in the Examination of Distinctness, Uniformity and Stability (DUS)" for the inclusion of a new model "Genetic selection of similar varieties for the first growing cycle", as follows:

New Section 2.3 "Genetic Selection of Similar Varieties for the First Growing Cycle"

2.3 Genetic Selection of Similar Varieties for the First Growing Cycle (see Annex III)

2.3.1 This approach involves a step to check for genetic similarity before the first growing cycle.

2.3.2 In cases where the minimum duration of tests is normally two growing cycles, a selection of similar varieties in the variety collection for comparison with candidate varieties in the first growing cycle is made according to genetic similarity. As a next step, the information provided by the applicant in the Technical Questionnaire (TQ) is used to see if some of the genetically similar varieties do not have to be compared in a growing trial because of differences in DUS characteristics.

2.3.3 On the basis of the variety description of DUS characteristics produced in the first growing cycle, a further search is made of varieties in the variety collection to identify any similar varieties that were not compared in the first growing cycle and which should be compared with the candidate variety in the second growing cycle.

2.3.4 Annex III to this document "Genetic Selection of Similar Varieties for the First Growing Cycle" provides an example of the genetic selection of similar varieties for the first growing cycle.

ANNEX III "MODEL: GENETIC SELECTION OF SIMILAR VARIETIES FOR THE FIRST GROWING CYCLE"

EXAMPLE: FRENCH BEAN

prepared by an expert from the Netherlands

1. Introduction

1.1 This approach involves a step to check for genetic similarity before the first growing cycle.

1.2 In cases where the minimum duration of tests is normally two growing cycles, a selection of similar varieties in the variety collection for comparison with candidate varieties in the first growing cycle is made according to genetic similarity. As a next step, the information provided by the applicant in the Technical Questionnaire (TQ) is used to see if some of the genetically similar varieties do not have to be compared in a growing trial because of differences in DUS characteristics.

1.3 On the basis of the variety description of DUS characteristics produced in the first growing cycle, a further search is made of varieties in the variety collection to identify any similar varieties that were not compared in the first growing cycle and which should be compared with the candidate variety in the second growing cycle.

2. Procedure

Determine genetic similarity

- 2.1 The DNA-profile of the candidate variety is produced as soon as plant material is received.
- 2.2 The DNA-profile is compared with the profiles of all varieties in the variety collection and genetically similar varieties are identified.

Technical Questionnaire information

- 2.3 The information provided by the applicant in the Technical Questionnaire (TQ) is then used to see if there are clear differences in DUS characteristics from some of the genetically similar varieties so that they do not need to be compared with candidate varieties in a growing trial.

Field trial

First growing cycle:

- 2.4 The candidate and the genetically similar varieties selected by the procedure above are grown in the same field trial. A complete description of the DUS characteristics of the candidate variety is produced and is compared to the descriptions of all varieties in the variety collection using a database containing descriptions produced at the same location in previous years.

- 2.5 Possible outcomes:

If the candidate variety is not distinct from the genetically similar varieties on the basis of DUS characteristics, the test will be continued for another growing cycle.

In any case, the description of the candidate variety produced in the first growing cycle is compared to the descriptions of the varieties in the variety collection using a database containing descriptions produced at the same location.

(a) If the candidate variety is found to be distinct from all varieties grown in the first growing cycle and to all other varieties in the variety collection at the end of the first growing cycle and it fulfills the uniformity and stability requirements the DUS test may be concluded after the first growing cycle.

(b) In all other cases a second growing cycle is performed.

Second growing cycle

- 2.6 In the second growing cycle, the candidate variety is grown with the all varieties in the variety collection from which it was not found to be distinct at the end of the first growing cycle.

- 2.7 At the end of the second growing cycle, an assessment of DUS is made. If it is not possible to reach a decision on DUS at the end of the second growing cycle, a further growing cycle may be conducted.

[Annex II follows]

EXAMPLE 2: GENE SPECIFIC MARKER FOR DISEASE RESISTANCE IN TOMATO

prepared by experts from The Netherlands

Example

1. Resistance to Tomato mosaic virus (ToMV) is included in the Test Guidelines for Tomato.
2. Resistance to ToMV Strain 0 is conferred by the presence of one or more genes, including Tm1; Tm2; and Tm2².

Table 1: Schematic overview of resistance to Tomato mosaic virus and resistance alleles:

Genetic background	tm2/tm2 and tm1/tm1	Tm2/Tm2 or Tm2/tm2 and Tm1/Tm1 or Tm1/tm1 or tm1/tm1	Tm2 ² /Tm2 ² or Tm2 ² /Tm2 or Tm2 ² /tm2 and Tm1/Tm1 or Tm1/tm1 or tm1/tm1	tm2tm2 and Tm1/Tm1 or Tm1/tm1
Marker Tm2/2 ²	susceptible allele	resistant allele	resistant allele	susceptible allele
Resistance to ToMV - Strain 0	absent	present	present	present

3. A marker identifies the presence of resistance alleles Tm2 and Tm2² and the susceptible allele tm2. Marker Tm2/2² is positioned in the protein coding sequence.
4. A variety will be resistant to ToMV Strain 0 if resistance allele Tm2 or resistance allele Tm2² is present. In this case, the DNA marker test could replace the traditional bioassay to assess resistance to ToMV Strain 0.
5. A variety with homozygous allele tm2 will be susceptible to ToMV Strain 0 unless resistance is coded by resistance allele Tm1. In this case, resistance to ToMV Strain 0 cannot be assessed by a DNA marker test because there is no reliable marker for gene Tm1.
6. If a variety is claimed to be resistant to ToMV Strain 0 and the DNA marker test result is tm2/tm2 (homozygous susceptible) a bioassay needs to be performed to determine whether the variety is resistant on the basis of another gene, such as Tm1.

[End of Annex II and of document]