



PROTOCOL FOR TESTS ON DISTINCTNESS, UNIFORMITY AND STABILITY

***Medicago sativa* L.
Medicago x varia Martyn**

**Lucerne, Alfalfa
Hybrid Lucerne**

UPOV Code: MEDIC_SAT_SAT; MEDIC_SAT_VAR

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1. SUBJECT OF THE PROTOCOL AND REPORTING

1.1 Scope of the technical protocol

This Technical Protocol applies to all varieties of *Medicago sativa* L. and *Medicago x varia* Martyn

The protocol describes the technical procedures to be followed in order to meet the requirements of Council Regulation 2100/94 on Community Plant Variety Rights. The technical procedures have been agreed by the Administrative Council and are based on documents agreed by the International Union for the Protection of New Varieties of Plants (UPOV), such as the General Introduction to DUS (UPOV Document TG/1/3 http://www.upov.int/export/sites/upov/resource/en/tg_1_3.pdf), its associated TGP documents (<http://www.upov.int/tgp/en/>) and the relevant UPOV Test Guideline TG/006/05 dated 06/04/2005 (<https://www.upov.int/edocs/tgdocs/en/tg006.pdf>) for the conduct of tests for Distinctness, Uniformity and Stability.

1.2 Entry into Force

The present protocol enters into force on **01.01.2022**. Any ongoing DUS examination of candidate varieties started before the aforesaid date will not be affected by the approval of the Technical Protocol. Technical examinations of candidate varieties are carried out according to the TP in force when the DUS test starts. The starting date of a DUS examination is considered to be the due date for submitting of plant material for the first test period.

In cases where the Office requests to take-over a DUS report for which the technical examination has either been finalized or which is in the process to be carried out at the moment of this request, such report can only be accepted if the technical examination has been carried out according to the CPVO TP which was in force at the moment when the technical examination started.

1.3 Reporting between Examination Office and CPVO and Liaison with Applicant

1.3.1 Reporting between Examination Office and CPVO

The Examination Office shall deliver to the CPVO a preliminary report ("the preliminary report") no later than two weeks after the date of the request for technical examination by the CPVO.

The Examination Office shall also deliver to the CPVO a report relating to each growing period ("the interim report") and, when the Examination Office considers the results of the technical examination to be adequate to evaluate the variety or the CPVO so requests, a report relating to the examination ("the final report").

The final report shall state the opinion of the Examination Office on the distinctness, uniformity and stability of the variety. Where it considers those criteria to be satisfied, or where the CPVO so requests, a description of the variety shall be added to the report. If a report is negative the Examination Office shall set out the detailed reasons for its findings.

The interim and the final reports shall be delivered to the CPVO as soon as possible and no later than on the deadlines as laid down in the designation agreement.

1.3.2 Informing on problems in the DUS test

If problems arise during the course of the test the CPVO should be informed immediately so that the information can be passed on to the applicant. Subject to prior permanent agreement, the applicant may be directly informed at the same time as the CPVO particularly if a visit to the trial is advisable.

1.3.3 Sample keeping in case of problems

If the technical examination has resulted in a negative report, the CPVO shall inform the Examination Office as soon as possible in case that a representative sample of any relevant testing material shall be kept.

2. MATERIAL REQUIRED

2.1 Plant material requirements

Information with respect to the agreed closing dates and submission requirements of plant material for the technical examination of varieties can be found on <https://public.plantvarieties.eu/publication> in the special issue S2/S3 of the Official Gazette of the Office. General requirements on submission of samples are also to be found following the same link.

2.2 Informing the applicant of plant material requirements

The CPVO informs the applicant that

- he is responsible for ensuring compliance with any customs and plant health requirements.
- the plant material supplied should be visibly healthy, not lacking in vigour, nor affected by any important pest or disease.
- the plant material should not have undergone any treatment which would affect the expression of the characteristics of the variety, unless the competent authorities allow or request such treatment. If it has been treated, full details of the treatment must be given.

2.3 Informing about problems on the submission of material

The Examination Office shall report to the CPVO immediately in cases where the test material of the candidate variety has not arrived in time or in cases where the material submitted does not fulfil the conditions laid down in the request for material issued by the CPVO.

In cases where the examination office encounters difficulties to obtain plant material of reference varieties the CPVO should be informed.

3. METHOD OF EXAMINATION

3.1 Number of growing cycles

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

3.2 Testing Place

Tests are normally conducted at one place. In the case of tests conducted at more than one place, guidance is provided in TGP/9 "Examining Distinctness" http://www.upov.int/edocs/tgpdocs/en/tgp_9.pdf

3.3 Conditions for Conducting the Examination

The tests should be carried out under conditions ensuring satisfactory growth for the expression of the relevant characteristics of the variety and for the conduct of the examination.

3.4 Test design

Each test should be designed to result in a total of at least 60 single spaced plants which should be divided between at least 3 replicates.

Unless otherwise indicated, all observations on single plants should be made on 60 plants or parts taken from each of 60 plants and any other observations made on all plants in the test. In the case of observations of parts taken from single plants, the number of parts to be taken from each of the plants should be 1.

The design of the test should be such that plants or parts of plants may be removed for measuring and counting without prejudice to the observations which must be made up to the end of the growing cycle.

3.5 Special tests for additional characteristics

In accordance with Article 23 of Implementing Rules N° 874/2009 an applicant may claim either in the Technical Questionnaire or during the test that a candidate has a characteristic which would be helpful in establishing distinctness. If such a claim is made and is supported by reliable technical data, a special test may be undertaken providing that a technically acceptable test procedure can be devised.

Special tests will be undertaken, with the agreement of the President of CPVO, where distinctness is unlikely to be shown using the characters listed in the protocol.

3.6 Constitution and maintenance of a variety collection

The process for the constitution and the maintenance of a variety collection can be summarized as follows:

Step 1: Making an inventory of the varieties of common knowledge

Step 2: Establishing a collection ("variety collection") of varieties of common knowledge which are relevant for the examination of distinctness of candidate varieties

Step 3: Selecting the varieties from the variety collection which need to be included in the growing trial or other tests for the examination of distinctness of a particular candidate variety.

3.6.1 Forms of variety collection

The variety collection shall comprise variety descriptions and living plant material, thus a living reference collection. The variety description shall be produced by the EO unless special cooperation exists between EOs and the CPVO. The descriptive and pictorial information produced by the EO shall be held and maintained in a form of a database.

3.6.2 Living Plant Material

The EO shall collect and maintain living plant material of varieties of the species concerned in the variety collection.

3.6.3 Range of the variety collection

The living variety collection shall cover at least those varieties that are suitable to climatic conditions of a respective EO."

3.6.4 Making an inventory of varieties of common knowledge for inclusion in the variety collection

The inventory shall take into account the list of protected varieties and the official, or other, registers of varieties, in particular:

The inventory shall include varieties protected under National PBR (UPOV contracting parties) and Community PBR, varieties registered in the Common Catalogue, the OECD list, the Conservation variety list and varieties in trade or in commercial registers for those species not covered by a National or the Common Catalogue."

3.6.5 Maintenance and renewal/update of a living variety collection

The EO shall maintain seeds in conditions which will ensure germination and viability, periodical checks, and renewal as required. For the renewal of existing living material the identity of replacement living plant material shall be verified by conducting side-by-side plot comparisons between the material in the collection and the new material.

4. ASSESSMENT OF DISTINCTNESS, UNIFORMITY AND STABILITY

The prescribed procedure is to assess distinctness, uniformity and stability in a growing trial.

4.1 Distinctness

4.1.1 General recommendations

It is of particular importance for users of this Technical Protocol to consult the UPOV-General Introduction to DUS (link in chapter 1 of this document) and TGP 9 'Examining Distinctness' (http://www.upov.int/edocs/tgpdocs/en/tgp_9.pdf) prior to making decisions regarding distinctness. However, the following points are provided for elaboration or emphasis in this Technical Protocol.

4.1.2. Consistent differences

The differences observed between varieties may be so clear that more than one growing cycle is not necessary. In addition, in some circumstances, the influence of the environment is not such that more than a single growing cycle is required to provide assurance that the differences observed between varieties are sufficiently consistent. One means of ensuring that a difference in a characteristic, observed in a growing trial, is sufficiently consistent is to examine the characteristic in at least two independent growing cycles.

4.1.3 Clear differences

Determining whether a difference between two varieties is clear depends on many factors, and should consider, in particular, the type of expression of the characteristic being examined, i.e. whether it is expressed in a qualitative, quantitative, or pseudo-qualitative manner. Therefore, it is important that users of these Technical Protocols are familiar with the recommendations contained in the UPOV-General Introduction to DUS prior to making decisions regarding distinctness.

If distinctness is assessed using the 2 x 1% criterion, the difference between two varieties is clear if the respective characteristics are significantly different in the same direction at the 1% level in at least two out of three growing cycles. The tests in each year are based on Student's two-tailed t-test of the differences between variety means with standard errors estimated using the residual mean square from the analysis of the variety x replicate plot means.

If distinctness is assessed by the combined over years distinctness analysis (COYD) the difference between two varieties is clear if the respective characteristics are different at the 1% significance level or less ($p < 0.01$) in a test over either two or three cycles.

If distinctness is assessed by the Pearson's Chi-Square test, the difference between two varieties is clear if the respective characteristics are significantly different in the same direction at the 1% level in at least two out of three growing cycles.

If the significance level or statistical methods indicated are not appropriate the method used should be clearly described.

4.1.4 Number of plants/parts of plants to be examined

Unless otherwise indicated, for the purposes of distinctness, all observations on single spaced plants should be made on 60 plants or parts taken from each of 60 plants in the spaced plants plots. In the case of observations of parts taken from single plants, the number of parts to be taken from each of the plants should be 1.

4.1.5 Method of observation

The recommended method of observing the characteristic for the purposes of distinctness is indicated by the following key in the third column of the Table of Characteristics (see document TGP/9 "Examining Distinctness", Section 4 "Observation of characteristics"):

MG:	single measurement of a group of plants or parts of plants
MS:	measurement of a number of individual plants or parts of plants
VG:	visual assessment by a single observation of a group of plants or parts of plants
VS:	visual assessment by observation of individual plants or parts of plants

Type of observation: visual (V) or measurement (M)

"Visual" observation (V) is an observation made on the basis of the expert's judgment. For the purposes of this document, "visual" observation refers to the sensory observations of the experts and, therefore, also includes smell, taste and touch. Visual observation includes observations where the expert uses reference points (e.g. diagrams, example varieties, side-by-side comparison) or non-linear charts (e.g. colour charts). Measurement (M) is an objective observation against a calibrated, linear scale e.g. using a ruler, weighing scales, colorimeter, dates, counts, etc.

Type of record: for a group of plants (G) or for single, individual plants (S)

For the purposes of distinctness, observations may be recorded as a single record for a group of plants or parts of plants (G), or may be recorded as records for a number of single, individual plants or parts of plants (S). In most cases, "G" provides a single record per variety and it is not possible or necessary to apply statistical methods in a plant-by-plant analysis for the assessment of distinctness."

In cases where more than one method of observing the characteristic is indicated in the Table of Characteristics (e.g. VG/MG), guidance on selecting an appropriate method is provided in document TGP/9, Section 4.2.

4.2 **Uniformity**

It is of particular importance for users of this Technical Protocol to consult the UPOV-General Introduction to DUS (link in chapter 1 of this document) and TGP 10 'Examining Uniformity' (http://www.upov.int/edocs/tgpdocs/en/tgp_10.pdf) prior to making decisions regarding uniformity. However, the following points are provided for elaboration or emphasis in this Technical Protocol:

The assessment of uniformity should be according to the recommendations for cross-pollinated varieties in the UPOV-General Introduction to DUS.

If uniformity is assessed by the combined over years uniformity method (COYU) the candidate variety is sufficiently uniform in the respective characteristic if the relative tolerance limit in relation to comparable varieties does not exceed the 1% significance level or less ($p < 0.01$) in a test over two years.

If uniformity is assessed by the combined over years uniformity method (COYU) the candidate variety is sufficiently uniform in the respective characteristic if the relative tolerance limit in relation to comparable varieties does not exceed the 0.1% significance level or less ($p < 0.001$) in a test over three years.

4.3 Stability

It is of particular importance for users of this Technical Protocol to consult the UPOV-General Introduction to DUS (link in chapter 1 of this document) and TGP 11 'Examining Stability' (http://www.upov.int/edocs/tgpdocs/en/tgp_11.pdf)

In practice, it is not usual to perform tests of stability that produce results as certain as those of the testing of distinctness and uniformity. However, experience has demonstrated that, for many types of variety, when a variety has been shown to be uniform, it can also be considered to be stable.

Where appropriate, or in cases of doubt, stability may be further examined by testing a new seed stock to ensure that it exhibits the same characteristics as those shown by the initial material supplied.

5. GROUPING OF VARIETIES AND ORGANIZATION OF THE GROWING TRIAL

5.1 The selection of varieties of common knowledge to be grown in the trial with the candidate varieties and the way in which these varieties are divided into groups to facilitate the assessment of distinctness are aided by the use of grouping characteristics.

5.2 Grouping characteristics are those in which the documented states of expression, even where produced at different locations, can be used, either individually or in combination with other such characteristics: (a) to select varieties of common knowledge that can be excluded from the growing trial used for examination of distinctness; and (b) to organize the growing trial so that similar varieties are grouped together.

5.3 The following have been agreed as useful grouping characteristics.

- a) Flower: frequency of plants with white to cream flowers (characteristic 1)
- b) Flower: frequency of plants with yellow flowers (characteristic 2)
- c) Flower: frequency of plants with light blue violet flowers (characteristic 3)
- d) Flower: frequency of plants with yellow and pink or yellow and green bicoloured flowers (characteristic 4)
- e) Flower: frequency of plants with light blue and violet bicoloured flowers (characteristic 5)
- f) Flower: frequency of plants with violet flowers (characteristic 6)
- g) Flower: frequency of plants with dark violet flowers (characteristic 7)
- h) Flower: frequency of plants very dark violet to purple flowers (characteristic 8)
- i) Flower: frequency of plants with dark blue and violet bicoloured flowers (characteristic 9)

5.4 If other characteristics than those from the TP are used for the selection of varieties to be included into the growing trial, the EO shall inform the CPVO and seek the prior consent of the CPVO before using these characteristics.

5.5 Guidance for the use of grouping characteristics, in the process of examining distinctness, is provided through the UPOV-General Introduction to DUS and document TGP/9 "Examining Distinctness".

6. INTRODUCTION TO THE TABLE OF CHARACTERISTICS

6.1 Characteristics to be used

The characteristics to be used in DUS tests and preparation of descriptions shall be those referred to in the table of characteristics. All the characteristics shall be used, providing that observation of a characteristic is not rendered impossible by the expression of any other characteristic, or the expression of a characteristic is prevented by the environmental conditions under which the test is conducted or by specific legislation on plant health. In the latter case, the CPVO should be informed.

The Administrative Council empowers the President, in accordance with Article 23 of Commission Regulation N°874/2009, to insert additional characteristics and their expressions in respect of a variety.

Technical Protocols with asterisked characteristics

In the case of disease resistance characteristics, only those resistances marked with an asterisk (*) in the CPVO column are compulsory.

States of expression and corresponding notes

In the case of qualitative and pseudo-qualitative characteristics, all relevant states of expression are presented in the characteristic. However, in the case of quantitative characteristics with 5 or more states, an abbreviated scale may be used to minimize the size of the Table of Characteristics. For example, in the case of a quantitative characteristic with 9 states, the presentation of states of expression in the Test Guidelines may be abbreviated as follows:

State	Note
Small	3
Medium	5
Large	7

However, it should be noted that all of the following 9 states of expression exist to describe varieties and should be used as appropriate:

State	Note
very small	1
very small to small	2
Small	3
small to medium	4
Medium	5
medium to large	6
Large	7
large to very large	8
very large	9

6.2 Example Varieties

Where appropriate, example varieties are provided to clarify the states of expression of each characteristic.

The example varieties provided are *Medicago sativa* except where indicated by "(M.v)" for *Medicago x varia* Martyn varieties.

6.3 Legend

For the CPVO N° column:

G	Grouping characteristic	– see Chapter 5
(*)	Asterisked characteristic	– see Chapter 6.1
QL	Qualitative characteristic	
QN	Quantitative characteristic	
PQ	Pseudo-qualitative characteristic	
(+)	Explanations for individual characteristics	- see Chapter 8.2

For the UPOV N° column:

The numbering of the characteristics is provided as a reference to the ad hoc UPOV guideline.

(*) UPOV Asterisked characteristic – Characteristics that are important for the international harmonization of variety descriptions.

For the column "stage, method":

10 - 69	Explanation on growth stages	- see Chapter 8.3
MG, MS, VG, VS	Method of observation	– see Chapter 4.1.5
(a) – (c)	Explanations covering several characteristics	–see chapter 8.1

7. TABLE OF CHARACTERISTICS

CPVO N°	UPOV N°	Stage, Method	Characteristics	Example varieties	Note
1.		63-65	Flower: frequency of plants with white to cream flowers		
QN	VS		absent or very low	Excelle, Juurlu (M.v.)	1
	(b)		Low		3
	(c)		medium		5
			high	Katana	7
G			very high		9
2.		63-65	Flower: frequency of plants with yellow flowers		
QN	VS		absent or very low	Excelle	1
	(b)		Low	Lucrezia	3
	(c)		medium	Juurlu (M.v.)	5
			high	Karlu (M.v.)	7
G			very high		9
3.		63-65	Flower: frequency of plants with light blue violet flowers		
QN	VS		absent or very low	Orca, Ulstar	1
	(b)		Low	Live (M.v.), Sibemol	3
	(c)		medium	Kometa (M.v.), Volga	5
			high	Carelite	7
G			very high		9
4.		63-65	Flower: frequency of plants with yellow and pink or yellow and green bicoloured flowers		
QN	VS		absent or very low	Excelle, Live (M.v.)	1
	(b)		low	Heiti (M.v.), Skriveru (M.v.)	3
	(c)		medium	Juurlu (M.v.), Karlu (M.v.)	5
			high		7
G			very high		9

CPVO N°	UPOV N°	Stage, Method	Characteristics	Example varieties	Note
5.		63-65	Flower: frequency of plants with light blue and violet bicoloured flowers		
QN	VS		absent or very low	Neptune	1
	(b)		low	Franken Neu, Jogeva 118 (M.v.), Sibemol	3
	(c)		medium		5
			high		7
G			very high		9
6.		63-65	Flower: frequency of plants with violet flowers		
QN	VS		absent or very low	Karlu (M.v.), Katana	1
	(b)		low	Kometa (M.v.), Nectarine, Vela	3
	(c)		medium	Greenmed	5
			high		7
G			very high		9
7.		63-65	Flower: frequency of plants with dark violet flowers		
QN	VS		absent or very low	Greenmed, Karlu (M.v.)	1
	(b)		low	Alexis, Litava	3
	(c)		medium	Joszo	5
			high		7
G			very high		9
8.		63-65	Flower: frequency of plants very dark violet to purple flowers		
QN	VS		absent or very low	Excele, Karlu (M.v.)	1
	(b)		low	Mnogolistna 1	3
	(c)		medium		5
			high	Orca	7
G			very high		9

CPVO N°	UPOV N°	Stage, Method	Characteristics	Example varieties	Note
9.		63-65	Flower: frequency of plants with dark blue and violet bicoloured flowers		
QN		VS	absent or very low	Excele, Karlu (M.v.)	1
		(b)	low	Heiti (M.v.), Vernal	3
		(c)	medium		5
			high		7
G			very high		9
10.	1.	5-39	Plant: growth habit in autumn of the first year (2 weeks before equinox)		
QN		VS	erect	KM Maraton, Körös 1	1
		(a)	semi erect	Jozso	3
			medium	Kakai legelő	5
			semi prostrate	Szentesi rona	7
			prostrate		9
11.	2. (*)	39-50	Plant: natural height 2 weeks after the first autumn equinox following sowing (cut 2 weeks before equinox)		
QN		MS	short	Karlu (M.v.), Luzelle	3
		(a)	medium	Andela, Fauna	5
			tall	Magali	7
12.	3. (*)	50-55	Plant: natural height 6 weeks after the first autumn equinox following sowing (cut 2 weeks after equinox)		
QN		MS	short	Boja	3
		(a)	medium	Diane	5
			Tall	Medalfa	7
13. (+)	4.	50-55	Plant: natural height in spring (1 month after beginning of growing the year after sowing)		
QN		MS	short	Karlu (M.v.), Vertus	3
			medium	Diane, Rival	5
			tall	Letizia, Magali	7

CPVO N°	UPOV N°	Stage, Method	Characteristics	Example varieties	Note
14. (+)	5. (*)		Time of beginning of flowering (the year after sowing)		
QN		MS	early	Alize	3
			medium	Luzelle	5
			late	Karlu (M.v.)	7
15. (+)	9. (*)	65	Stem: length of the longest stem at full flowering (head included; when fully expanded)		
QN		MS	short	Karlu (M.v.)	3
			medium	Carmen, Franken Neu	5
			long	Fauna	7
16. (+)	10.	50	Plant: natural height 3 weeks after 1st cut		
QN		MS	short	Karlu (M.v.)	3
			medium	Andela, Symphonie	5
			tall	Zenith	7
17. (+)	11.	50	Plant: natural height 3 weeks after 2nd cut		
QN		MS	short	Karlu (M.v.)	3
			medium	Andela, Franken Neu	5
			tall	Zenith	7
18. (+)	12.	39-50	Plant: natural height 3 weeks after 3rd cut		
QN		MS	short	Karlu (M.v.)	3
			medium	Timbale	5
			tall	Letizia, Zenith	7
19. (+)	13.	50	Plant: natural height 3 weeks after 4th cut		
QN		MS	short	Karlu (M.v.)	3
			medium	Andela, Symphonie	5
			tall	Carmen, Zenith	7

CPVO N°	UPOV N°	Stage, Method	Characteristics	Example varieties	Note		
20.	14.	39-50	Plant: natural height 2 weeks after the second autumn equinox following sowing (cut 2 weeks before equinox)				
			QN	MS	short	Gibraltar	3
					(a)	medium	Fauna
	tall	Zenith			7		
21.	15.	50-55	Plant: natural height 6 weeks after the second autumn equinox following sowing (cut 2 weeks after equinox)				
			QN	MS	short	Boja	3
					(a)	medium	Europe
	tall	Zenith			7		
22. (+)	16. (*)	MG	Plant: tendency to grow during winter				
			QN	VG	Dormancy rating 1	Juurlu (M.v.), Heiti (M.v.)	1
					Dormancy rating 2	Luzelle, Vernal	2
					Dormancy rating 3	Musette, Nectarine	3
					Dormancy rating 4	Asmara, Sibemol	4
					Dormancy rating 5	Prista 4, RGT Dentelle	5
					Dormancy rating 6	Medoc, Nogara	6
					Dormancy rating 7	Sutter, Tequilla	7
					Dormancy rating 8	Maricopa, Verdor	8
					Dormancy rating 9	Alcor, Excellente Multileaf	9
					Dormancy rating 10	Cuf 101, Escorial	10
Dormancy rating 11		11					

CPVO N°	UPOV N°	Stage, Method	Characteristics	Example varieties	Note
23. (+) QN	17.	VS	Tolerance to <i>Verticillium albo-atrum</i>		
			low	Magali, Medalfa	3
			medium	Derby, Europe	5
			high	Vertus	7
24. (+) QN	18.	VS	Tolerance to <i>Ditylenchus dipsaci</i>		
			very low		1
			low	Europe	3
			medium	Daisy, Midi	5
		high	Salsa, Vertus	7	
25. (+) QN	19.	VS	Tolerance to <i>Colletotrichum trifolii</i>		
			very low	Kali	1
			low	Venus	3
			medium		5
			high	Ambra, Artémis	7
		very high		9	

8. EXPLANATIONS ON THE TABLE OF CHARACTERISTICS

8.1 Explanations covering several characteristics

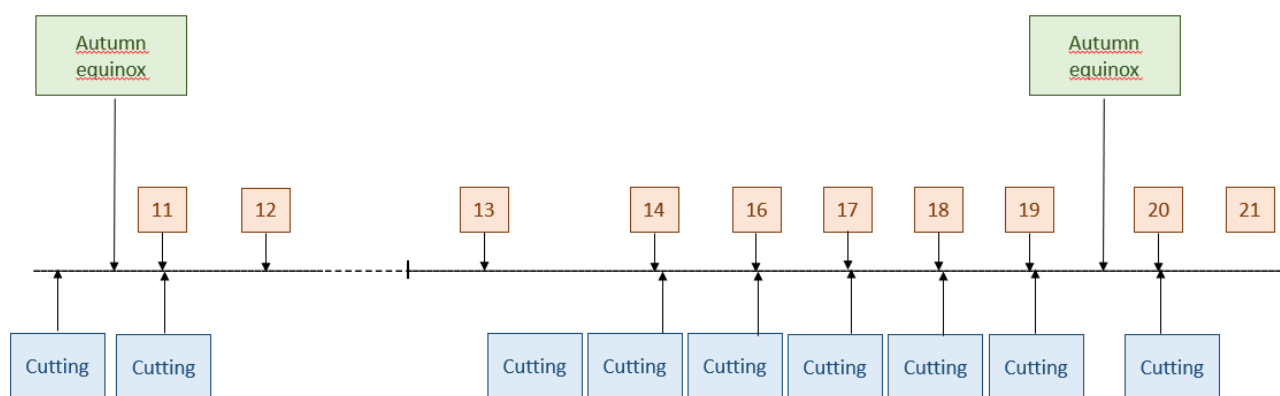
Characteristics containing the following key in the first column of the Table of Characteristics should be examined as indicated below

- a) The equinox referred to in characteristics 11, 12, 20 and 21 and elsewhere in the text refers to the autumnal or fall equinox. This occurs on September 22 for the Northern hemisphere. It is an appropriate date on which to base the plant height measurements relating to the degree of fall dormancy.

In Characteristics 11 and 12, the plant height measurements should be respectively taken 2 and 6 weeks after the first autumnal equinox.

In Characteristics 20 and 21, the plant height measurements should be respectively taken 2 and 6 weeks after the second autumnal equinox.

The following diagram shows the time of the year when these measurements should be taken.



- b) Observations on flower colour should be made at the beginning of flowering on young flowers (2 to 3 days after opening) using RHS code 2015. The frequency should be assessed on spaced plants (A). The states of expression cover the whole range from 1% to 100% irrespective of the actual range in the existing varieties. Variegation is defined by the presence of yellow and violet pigments within the same flower. This combination may give the appearance of bicoloured flowers.

Characteristic	RHS class
<u>Ad. 1: Flower: frequency of plants with white to cream flowers</u>	white group 155 and NN155
<u>Ad. 2: Flower: frequency of plants with yellow flowers</u>	yellow group 2 to 10
<u>Ad. 3: Flower: frequency of plants with light blue violet flowers</u>	violet blue group 91 and 92
<u>Ad. 4: Flower: frequency of plants with yellow and pink or yellow and green bicoloured flowers</u>	yellow-green group 144, N144, 145 and 146 mixed with yellow group 2 to 10, as well as red-purple group 62 and 65 mixed with yellow group 2 to 10
<u>Ad. 5: Flower: frequency of plants with light blue and violet bicoloured flowers</u>	violet group 90 to 92 mixed with blue group 104 to 106
<u>Ad. 6: Flower: frequency of plants violet flowers</u>	violet group 90
<u>Ad. 7: Flower: frequency of plants with dark violet flowers</u>	violet group 86 and violet blue group 90
<u>Ad. 8: Flower: frequency of plants with very dark violet to purple flowers</u>	violet group N88, 83 and red purple group 71
<u>Ad. 9: Flower: frequency of plants with dark blue and violet bicoloured flowers</u>	violet group N88, 83 and red purple group 71 mixed with blue group 102, 103 and 105

- c) Where appropriate for analysis with specific statistical tests like Chi-square and Fischer exact tests, in each growing cycle, a special test for flower colour should be designed to result in a total of at least 72 single spaced plants which should be divided between at least 4 replicates. The test should include the example varieties, candidate varieties and similar varieties.

The method is based on a study as described in UPOV/TWC/24/16.
https://www.upov.int/edocs/mdocs/upov/en/twc/24/twc_24_16.pdf).

8.2 Explanations for individual characteristics

Ad. 13: Plant: natural height in spring (1 month after beginning of growing the year after sowing)

The measurement should be done one month after the earliest varieties start to grow and reach about 15 cm height.

Ad. 14: Time of beginning of flowering (the year after sowing)

The date of beginning of flowering of each single plant should be assessed. A single plant is considered to have headed when three inflorescences can be seen (on three different stem, at least one flower per stem). From the single plant data a mean date per plot and a mean date per variety are obtained.

Ad. 16: Plant: natural height 3 weeks after 1st cut

The first cut should be done just after full flowering, when characteristic 15: "Stem: length of longest stem at full flowering (head included; when fully expanded)" has been assessed.

Ad.17: Plant: natural height 3 weeks after 2nd cut

The plants should be cut just after characteristic 16 "Plant: natural height 3 weeks after 1st cut" has been measured.

Ad.18: Plant: natural height 3 weeks after 3rd cut

The plants should be cut just after characteristic 17 "Plant: natural height 3 weeks after 2nd cut" has been measured.

Ad.19: Plant: natural height 3 weeks after 4th cut

The plants should be cut just after characteristic 18 "Plant, natural height 3 weeks after 3rd cut" has been measured.

Ad.22: Plant: tendency to grow during winter

This characteristic is also referred to as "dormancy" and indicates the growth rate in autumn (fall) and early spring in the northern hemisphere countries. Expression depends on the combination of shortening day length and cool temperatures. Under short day conditions, differences among dormant and non-dormant varieties are more pronounced at low temperatures. At cool temperatures, dormant varieties have the greatest dormancy response and non-dormant varieties have the lowest response. Under long day conditions, there is a little difference in regrowth between dormant and non-dormant varieties.

The characteristic should be assessed during the autumn period, but before a severe frost and/or the beginning of spring. Local experience will provide information on which cut date provides the greatest differentiation between varieties (Teuber *et al.*, 1998; Montegano *et al.*, 2002).

The characteristic can be easily recorded visually. The following table indicates the correspondence between the dormancy rating (see Teuber *et al.*, 1998; Montegano *et al.*, 2002) and the notes in the Table of Characteristics:

Example varieties	Dormancy rating (Teuber <i>et al.</i> , 1998; Montegano <i>et al.</i> , 2002)	Note (Table of Characteristics)
Maverick	1	1
Vernal	2	2
Boja, Ranger	3	3
Legend, Mercedes, Cutter	4	4
Archer, Dupuits	5	5
Abi 700, Dorine	6	6
Sutter, Oro, Dona Ana	7	7
Maricopa, Carmen, 5715	8	8
CUF 101, Medina, 5929	9	9
UC-1887	10	10
UC-1465	11	11

The dormancy score can also be calculated by considering height measurements of a group of plants (MG) during the dormancy period.

The following characteristics are then used:

- Char. 11: Plant: natural height 2 weeks after the first autumn equinox following sowing (cut 2 weeks before equinox)
- Char. 12: Plant: natural height 6 weeks after the first autumn equinox following sowing (cut 2 weeks after equinox)
- Char. 13: Plant: natural height in spring (1 month after beginning of growing the year after sowing)
- Char. 20: Plant: natural height 2 weeks after the second autumn equinox following sowing (cut 2 weeks before equinox)
- Char. 21: Plant: natural height 6 weeks after the second autumn equinox following sowing (cut 2 weeks after equinox)

The method is based on a linear regression model as described in the publication Montegano *et al.*, 2002 (see Chapter 9).

Ad.23: Tolerance to *Verticillium albo-atrum*

- The seeds are pre-germinated by sowing on wet blotting paper in Petri dishes.
- When the germs are 4 to 5 mm long, they should be transplanted to pots. (For example, 50 germs can be transplanted to a pot of 30 cm x 30 cm). It is recommended that 150 plants per variety be observed.
- The pots should be placed in a greenhouse at 20°C for three months. During one month, fertilizer should be provided (250 ml per pot and twice per week).

E.g. Fertilizer solution for 20 litres:

(NO ₃) ₂ CaH ₂ O	20g
NO ₃ K	5g
SO ₄ Mg ₇ H ₂ O	5g
PO ₄ H ₂ K	5g

- Optional: The plants can be cut between 2 to 3 cm and inoculated one month later.

5. The inoculum should be obtained after three weeks of culture made on e.g.

a. the following substrate:

Saccharose	20 g
Extract of crystallizable malt	5 g
Citric acid	25 mg
Malic acid	25 mg
Iron chelate	20 mg
SO ₄ Mn ₂ H ₂ O	3 mg
SO ₄ Cu ₅ H ₂ O	3 mg
H ₃ BO ₃	4 mg
SO ₄ Zn ₇ H ₂ O	3 mg
KNOP	solution made up to 1000 ml

After the inoculum has been ground with a mixer, the suspension should contain 10⁶ spores by mm³.

b. Or on a liquid media (Messiaen modified).

The suspension should be filtered or mixed and should contain between 10⁸ spores per mL to 10⁶ by mm³

6. Contamination is by clipping the plants down to between 4 and 5 cm from the crown with scissors that have previously been dipped into the suspension.
7. The pots are immediately covered or transferred to a chamber with a relative humidity of between 80 and 100%. Optional: The temperature should be 17°C to 20°C and the light intensity between 10,000 and 15,000 lux.
8. The observations should be made 30 days later. To each plant one of the following notes is attributed:
- 4 dried plant
 - 3 one stunted stem on the plant
 - 2 dried leaf
 - 1 enlightened veins
 - 0 absence of symptoms

For each variety, the mean is calculated from the total of the notes divided by the number of plants observed.

9. It is recommended that the following varieties have the appropriate notes to ensure that the descriptions are consistent:

Magali	low	3
Derby,	medium	5
Sitel, Lifeuil, Europe	medium to high	6
Vertus	high	7

Ad. 24: Tolerance to *Ditylenchus dipsaci*

- Seeds are abraded, disinfected (15 minutes in Metalaxyl 1g/L or 10 minutes in Hypochlorite 1%) and pregerminated by sowing them in vermiculite (2000 seeds are sown to have 300 seeds germinated). It is recommended that 300 plants per variety be observed.
- After 4-4.5 days at 18-19°C, photoperiod of 12-14 hours daylight, the seedling (the length of the root is nearly 1 cm) should be laid on soaked blotting paper of 240g (2 strips of 40 x 10 cm). The seedlings are placed within the central third on the upper part of the strip, only the cotyledons must not be on the paper. The two extremities of the upper strip are folded on the roots. The second strip of blotting paper is used for the roll up. For each variety 16 rolls of 20 seedlings are made. The rolls are deposited in pots of 30 x 30cm, with water (1 cm deep) one variety per pot.
- The pots should be put in a climatic chamber at 19°C, 12 hours of photoperiod (11 - 15,000 lux) and 80% humidity.
- Two days later, when the cotyledons are well opened, the inoculation is done with a micro pipette. On each seedling, deposit a drop of 20 micro litres containing 5-50 nematodes (depending of aggressivity of population) between the two cotyledons and mix with carbomethylcellulose at 40%. 15 rollers per genotype are inoculated.

5. Observations should be made between 14 and 21 days after the inoculation. To each plant one of the following expressions is attributed:
- puffed seedling (sensitive seedling)
 - dead seedling

For each variety, the percentage is calculated from the total of the number of puffed seedlings divided by the sum of puffed seedlings + stopped growth seedlings + seedlings without symptoms.

6. It is recommended that the following varieties have the appropriate notes to ensure that the descriptions are consistent:

Europe	low	3
Daisy, Midi	medium	5
Vertus, Salsa	high	7

Ad. 25: Tolerance to *Colletotrichum trifolii* Bain and Essary (Anthracnose)

(Based on standard test guidelines as published by the North American Alfalfa Improvement Conference)

Plant Culture:

Container	10 cm plastic pots or flats or 30*30cm plastic pots
Medium	Potting soil mix
Temp/Light	23°C; 16+ hour day length
No. of Plants	50 - 100 per replication
No. of Reps	3 - 4 minimum
Other	optional: Control insects and fertilize as necessary

Inoculum Culture:

Source	Infected stem tissue
Storage	Soil or silica gel (7)
Temperature	4°C
Storage Life	Up to several years
Or	
Source	Isolate race 1 GEVES ¹ (F)
Storage	Glycerol suspension
Temperature	-80°C
Storage Life	5 years

Inoculation Procedure:

Age of Plant	7-14 days (take stand counts at 7 days)
Type of Inoc.	Spore suspension with 2-3 drops Tween per litre distilled water, taken from 7 day old cultures incubated at 23°C on half strength oatmeal agar or 14 day old cultures incubated at 20°C on Potatoes Dextrose Agar (optional with antibiotics).
Concentration	2 x 10 ⁶ spores per ml
Method	Spray to runoff, approx. 3 ml or 5 to 10 ml per flat or 20 mL for 30*30cm plastic pots; covered or place in mist chamber to maintain 100% R.H. for 48 hours at 20-23°C

Incubation:

Location	Growth room or greenhouse at 20-23°C
Age at Rating	10 to 14 days after inoculation

State of Expression Example varieties (Race 1)

Highly resistant (>50%)	Sequel HR
Resistant (31-50%)	Trifecta
Moderately resistant (15-30%)	
Weakly resistant (6-14%)	Venus
Susceptible (0-<6%)	Hunter River

Or

Kali	very low	1
Alexis	very low to low	2
Marshall, Artemis	medium to high	6
Ambra	high to very high	7

Rating:

Resistance is assessed as percentage of seedlings surviving 10 to 14 days after inoculation.

Check varieties (Race 1):

¹ GEVES, contact@geves.fr

8.3 Explanation of growth stages

Phenological growth stages and BBCH-identification keys of Lucerne, adapted from BBCH of Pea

Principal growth stage 1: Leaf development

- 10 – First leaf visible
- 11 – First true leaf unfolded
- 12 – 2 leaves unfolded
- 13 – 3 leaves unfolded
- 1.. – Stages continuous till...
- 19 – 9 or more leaves unfolded

Principal growth stage 2: Stem development

- 20 – main stem only
- 21 – main stem and another 1
- 22 – main stem and another 2
- 23 – main stem and another 3
- 2.. – Stages continuous till...
- 29 – main stem and another 9 or more

Principal growth stage 3: Stem elongation

- 30 – Beginning of stem elongation
- 31 – 1 visibly extended internode
- 32 – 2 visibly extended internodes
- 33 – 3 visibly extended internodes
- 3.. – Stages continuous till...
- 39 – 9 or more extended internodes

Principal growth stage 5: Inflorescence emergence

- 50 – First flower buds visible (sporadically within the population)
- 55 – First separated flower buds visible outside leaves but still closed
- 59 – First petals visible, flowers still closed

Principal growth stage 6: Flowering

- 60 – First flowers open (sporadically within population)
- 61 – Beginning of flowering: 10% of flowers open
- 62 – 20% of flowers open
- 63 – 30% of flowers open
- 64 – 40% of flowers open
- 65 – Full flowering: 50% of flowers open
- 67 – Flowering declining
- 69 – End of flowering

9. LITERATURE

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10. TECHNICAL QUESTIONNAIRE

The Technical Questionnaire is available on the [CPVO website](#) under the following reference:
CPVO-TQ/006/1 – *Medicago sativa* L.; *Medicago x varia* Martyn – Lucerne, alfalfa, hybrid lucerne

<https://applyfor.plantvarieties.eu/myprv.oa/#!/en/oa/show/questionnaire/TQ/12917/en>