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INTERNATIONAL UNION FOR THE PROTECTION OF NEW VARIETIES OF PLANTS
GENEVA

DRAFT**LUCERNE**

UPOV code: MEDIC_SAT_SAT
MEDIC_SAT_VAR

*(Medicago sativa L. and
Medicago x varia Martyn)*

GUIDELINES**FOR THE CONDUCT OF TESTS****FOR DISTINCTNESS, UNIFORMITY AND STABILITY**

prepared by experts from France

*to be considered by the Technical Committee at its forty-first session,
to be held in Geneva, Switzerland, from April 4 to 6, 2005*

Alternative Names:*

<i>Botanical name</i>	<i>English</i>	<i>French</i>	<i>German</i>	<i>Spanish</i>
<i>Medicago sativa</i> L., <i>Medicago sativa</i> L. <i>subsp. sativa</i>	Lucerne, Alfalfa	Luzerne	Blaue Luzerne	Alfalfa, Mielga
<i>Medicago x varia</i> , Martyn, <i>Medicago sativa</i> L. <i>nothosubsp. varia</i> (Martyn) Arcang.	Hybrid Lucerne, Sand Lucerne Variegated Lucerne	Luzerne bigarrée Luzerne hybride Luzerne intermédiaire	Bastardluzerne Sandluzerne	Alfalfa de las arenas Alfalfa híbrida

The purpose of these guidelines ("Test 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 examination of distinctness, uniformity and stability (DUS) and, in particular, to identify appropriate characteristics for the examination of DUS and production of harmonized variety descriptions.

ASSOCIATED DOCUMENTS

These Test Guidelines should be read in conjunction with the General Introduction and its associated TGP documents.

* These names were correct at the time of the introduction of these Test Guidelines but may be revised or updated. [Readers are advised to consult the UPOV Code, which can be found on the UPOV Website (www.upov.int), for the latest information.]

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1. Subject of these Test Guidelines

These Test Guidelines apply to all varieties of *Medicago sativa* L. and *Medicago x varia* Martyn.

2. Material Required

2.1 The competent authorities decide on the quantity and quality of the plant material required for testing the variety and when and where it is to be delivered. Applicants submitting material from a State other than that in which the testing takes place must ensure that all customs formalities and phytosanitary requirements are complied with.

2.2 The material is to be supplied in the form of seed.

2.3 The minimum quantity of plant material, to be supplied by the applicant, should be:

1 kg.

The seed should meet the minimum requirements for germination, species and analytical purity, health and moisture content, specified by the competent authority. In cases where the seed is to be stored, the germination capacity should be as high as possible and should, be stated by the applicant.

2.4 The plant material supplied should be visibly healthy, not lacking in vigor, nor affected by any important pest or disease.

2.5 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.

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".

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.

The optimum stage of development for the assessment of each characteristic is indicated in the second column of the Table of Characteristics.

The recommended method of observing the characteristic is indicated by the following key in the second column of the Table 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

The recommended type of plot in which to observe the characteristic is indicated by the following key in the second column of the Table of Characteristics:

- A: spaced plants
- B: row plot
- C: special test

3.4 *Test Design*

3.4.1 Each test should be designed to result in a total of at least 60 spaced plants and 10 meters of row plot, which should be divided between 3 replicates.

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

3.5 *Number of Plants / Parts of Plants to be Examined*

Unless otherwise indicated, all observations on single spaced plants should be made on 60 plants or parts taken from each of 60 plants in the spaced plant plots.

3.6 *Additional Tests*

Additional tests, for examining relevant characteristics, may be established.

4. Assessment of Distinctness, Uniformity and Stability

4.1 *Distinctness*

4.1.1 General Recommendations

It is of particular importance for users of these Test Guidelines to consult the General Introduction prior to making decisions regarding distinctness. However, the following points are provided for elaboration or emphasis in these Test Guidelines.

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 Test Guidelines are familiar with the recommendations contained in the General Introduction prior to making decisions regarding distinctness.

4.2 *Uniformity*

4.2.1 It is of particular importance for users of these Test Guidelines to consult the General Introduction prior to making decisions regarding uniformity. However, the following points are provided for elaboration or emphasis in these Test Guidelines:

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

4.3 *Stability*

4.3.1 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.

4.3.2 Where appropriate, or in cases of doubt, stability may be tested, either by growing a further generation, or by testing a new seed stock to ensure that it exhibits the same characteristics as those shown by the previous 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 very dark blue violet flowers (characteristic 6)
- (b) Flower: frequency of plants with variegated flowers (characteristic 7)
- (c) Flower: frequency of plants with cream, white or yellow flowers (characteristic 8)

5.4 Guidance for the use of grouping characteristics, in the process of examining distinctness, is provided through the General Introduction.

6. Introduction to the Table of Characteristics

6.1 *Categories of Characteristics*

6.1.1 Standard Test Guidelines Characteristics

Standard Test Guidelines characteristics are those which are approved by UPOV for examination of DUS and from which members of the Union can select those suitable for their particular circumstances.

6.1.2 Asterisked Characteristics

Asterisked characteristics (denoted by *) are those included in the Test Guidelines which are important for the international harmonization of variety descriptions and should always be examined for DUS and included in the variety description by all members of the Union, except when the state of expression of a preceding characteristic or regional environmental conditions render this inappropriate.

6.2 *States of Expression and Corresponding Notes*

States of expression are given for each characteristic to define the characteristic and to harmonize descriptions. Each state of expression is allocated a corresponding numerical note for ease of recording of data and for the production and exchange of the description.

6.3 *Types of Expression*

An explanation of the types of expression of characteristics (qualitative, quantitative and pseudo-qualitative) is provided in the General Introduction.

6.4 *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.

6.5 Legend

(*) Asterisked characteristic– see Chapter 6.1.2

QL: Qualitative characteristic – see Chapter 6.3

QN: Quantitative characteristic – see Chapter 6.3

PQ: Pseudo-qualitative characteristic – see Chapter 6.3

MG: Single measurement of a group of plants or parts of plants – see Chapter 3.3

MS: Measurement of a number of individual plants or parts of plants – see Chapter 3.3

VG: Visual assessment by a single observation of a group of plants or parts of plants – see Chapter 3.3

VS: Visual assessment by observation of individual plants or parts of plants – see Chapter 3.3

(a)-(b) See Explanations on the Table of Characteristics in Chapter 8.1

(+) See Explanations on the Table of Characteristics in Chapter 8.2

(M.v) Example variety of *Medicago x varia* Martyn

7 Table of Characteristics/Tableau des caractères/Merkmalstabelle/Tabla de caracteres

	English	français	deutsch	español	Example Varieties/ Exemples/ Beispielssorten/ Variedades ejemplo	Note/ Nota	
1.	VG B	Plant: growth habit in autumn of the first year (2 weeks before equinox)	Plante: port à l'automne de la première année (deux semaines avant l'équinoxe)	Pflanze: Wuchsform im Herbst des ersten Jahres (2 Wochen vor der Tag- und Nachtgleiche)	Planta: porte en el otoño del primer año (2 semanas antes del equinoccio)		
QN	erect	dressé	aufrecht	erecto	KM Maraton, Körös 1	1	
	semi erect	demi-dressé	halbaufrecht	semierecto	Jozso	3	
	medium	demi-dressé à demi-étalé	mittel	medio	Kakai legelő	5	
	semi prostrate	demi-étalé	halbliiegend	semipostrado	Szentesi rona	7	
	prostrate	étalé	liegend	postrado		9	
2. (*)	MS A MG B	Plant: natural height 2 weeks after the first autumn equinox following sowing (cut 2 weeks before equinox)	Plante: hauteur naturelle deux semaines après le premier équinoxe d'automne suivant le semis (coupe deux semaines avant l'équinoxe)	Pflanze: Wuchshöhe 2 Wochen nach der ersten Herbst-Tag- und Nachtgleiche nach der Aussaat (Schnitt 2 Wochen vor der Tag- und Nachtgleiche)	Planta: altura 2 semanas después del primer equinoccio de otoño siguiente a la siembra (corte 2 semanas antes del equinoccio)		
QN	(a)	short	basse	niedrig	baja	Karlu (M.v.), Luzelle	3
	(b)	medium	moyenne	mittel	media	Andela, Fauna	5
		tall	haute	hoch	alta	Magali	7
3. (*)	MG B	Plant: natural height 6 weeks after the first autumn equinox following sowing (cut 2 weeks after equinox)	Plante: hauteur naturelle six semaines après le premier équinoxe d'automne suivant le semis (coupe deux semaines après l'équinoxe)	Pflanze: Wuchshöhe 6 Wochen nach der ersten Herbst-Tag- und Nachtgleiche nach der Aussaat (Schnitt 2 Wochen nach der Tag- und Nachtgleiche)	Planta: altura 6 semanas después del primer equinoccio de otoño siguiente a la siembra (corte 2 semanas después del equinoccio)		
QN	(a)	short	basse	niedrig	baja	Boja	3
	(b)	medium	moyenne	mittel	media	Diane	5
		tall	haute	hoch	alta	Medalfa	7

		English	français	deutsch	español	Example Varieties/ Exemples/ Beispielssorten/ Variedades ejemplo	Note/ Nota
4.	MS	Plant: natural height in spring	Plante: hauteur naturelle au printemps	Pflanze: Wuchshöhe im Frühjahr	Planta: altura en la primavera		
(+)	A	(1 month after beginning of growing the year after sowing)	(un mois après le début de la croissance l'année suivant le semis)	(1 Monat nach Beginn des Wachstums im Jahr nach der Aussaat)	(1 mes después del comienzo del crecimiento el año siguiente a la siembra)		
	MG						
	B						
QN	(b)	short	basse	niedrig	baja	Karlu (M.v.), Vertus	3
		medium	moyenne	mittel	media	Diane, Rival	5
		tall	haute	hoch	alta	Letizia, Magali	7
5.	MS	Time of beginning of flowering	Époque de début de floraison	Zeitpunkt des Blühbeginns	Época de comienzo de la floración		
(*)	A						
(+)	MG						
	B						
QN		early	précoce	früh	temprana	Alize	3
		medium	moyenne	mittel	media	Luzelle	5
		late	tardive	spät	tardía	Karlu (M.v.)	7
6.	VS	Flower:frequency of plants with very dark blue violet flowers	Fleur: fréquence des plantes à fleurs violet-bleu très foncé	Blüte: Häufigkeit von Pflanzen mit sehr dunkelblau-violetten Blüten	Flor: frecuencia de plantas con flores de color violeta azulado muy oscuro		
(*)	A						
QN	(c)	absent or very low	nulle ou très faible	fehlend oder sehr gering	ausente o muy baja	Diane	1
		low	faible	gering	baja	Sanditi	3
		medium	moyenne	mittel	media	Andela	5
		high	élevée	hoch	alta	Orca	7

		English	français	deutsch	español	Example Varieties/ Exemples/ Beispielsorten/ Variedades ejemplo	Note/ Nota
7.	VS	Flower: frequency of plants with variegated flowers	Fleur: fréquence des plantes à fleurs bigarrées	Blüte: Häufigkeit von Pflanzen mit mischfarbenen Blüten	Flor: frecuencia de plantas con flores variegadas		
(*)	A						
QN	(c)	absent or very low	nulle ou très faible	fehlend oder sehr gering	ausente o muy baja	Symphonie	1
		low	faible	gering	baja	Luzelle, Letizia	3
		medium	moyenne	mittel	media	Franken Neu, Karlu (M.v.)	5
		high	élevée	hoch	alta		7
8.	VS	Flower: frequency of plants with cream, white or yellow flowers	Fleur: fréquence des plantes à fleurs crème, blanches ou jaunes	Blüte: Häufigkeit von Pflanzen mit cremefarbenen, weißen oder gelben Blüten	Flor: frecuencia de plantas con flores de color crema, blanco o amarillo		
(*)	A						
QN	(c)	absent or very low	nulle ou très faible	fehlend oder sehr gering	ausente o muy baja	Europe	1
		low	faible	gering	baja		3
		medium	moyenne	mittel	mediana	Karlu (M.v.)	5
		high	élevée	hoch	alta		7
9.	MS	Stem: length of the longest stem at full flowering (head included; when fully expanded)	Tige: longueur de la tige la plus longue à l'époque de pleine floraison (inflorescence incluse; tige déployée)	Stengel: Länge des längsten Stengels bei Vollblüte (Blütenstand eingeschlossen; wenn vollständig ausgebildet)	Tallo: longitud del tallo más largo en plena floración (incluida la inflorescencia cuando está completamente abierta)		
(*)	A						
QN		short	courte	kurz	corto	Karlu (M.v.)	3
		medium	moyenne	mittel	medio	Franken Neu, Carmen	5
		long	longue	lang	largo	Fauna	7

		English	français	deutsch	español	Example Varieties/ Exemples/ Beispielsorten/ Variedades ejemplo	Note/ Nota
10.	MS	Plant: natural height 3 weeks after 1st cut	Plante: hauteur naturelle trois semaines après la première coupe	Pflanze: Wuchshöhe 3 Wochen nach dem ersten Schnitt	Planta: altura 3 semanas después del primer corte		
(+)	MG						
	B						
QN	(b)	short	basse	niedrig	baja	Karlu (M.v.)	3
		medium	moyenne	mittel	media	Andela, Symphonie	5
		tall	haute	hoch	alta	Zenith	7
11.	MS	Plant: natural height 3 weeks after 2nd cut	Plante: hauteur naturelle trois semaines après la deuxième coupe	Pflanze: Wuchshöhe 3 Wochen nach dem zweiten Schnitt	Planta: altura 3 semanas después del segundo corte		
(+)	MG						
	B						
QN	(b)	short	basse	niedrig	baja	Karlu (M.v.)	3
		medium	moyenne	mittel	media	Franken Neu, Andela	5
		tall	haute	hoch	alta	Zenith	7
12.	MS	Plant: natural height 3 weeks after 3rd cut	Plante: hauteur naturelle trois semaines après la troisième coupe	Pflanze: Wuchshöhe 3 Wochen nach dem dritten Schnitt	Planta: altura 3 semanas después del tercer corte		
(+)	MG						
	B						
QN	(b)	short	basse	niedrig	baja	Karlu (M.v.)	3
		medium	moyenne	mittel	media	Timbale	5
		tall	haute	hoch	alta	Letizia, Zenith	7
13.	MS	Plant: natural height 3 weeks after 4th cut	Plante: hauteur naturelle trois semaines après la quatrième coupe	Pflanze: Wuchshöhe 3 Wochen nach dem vierten Schnitt	Planta: altura 3 semanas después del cuarto corte		
(+)	MG						
	B						
QN		short	basse	niedrig	baja	Karlu (M.v.)	3
		medium	moyenne	mittel	media	Symphonie, Andela	5
		tall	haute	hoch	alta	Carmen, Zenith	7

	English	français	deutsch	español	Example Varieties/ Exemples/ Beispielssorten/ Variedades ejemplo	Note/ Nota	
14.	MG B	Plant: natural height 2 weeks after the second autumn equinox following sowing (cut 2 weeks before equinox)	Plante: hauteur naturelle deux semaines après le deuxième équinoxe d'automne suivant le semis (coupe deux semaines avant l'équinoxe)	Pflanze: Wuchshöhe 2 Wochen nach der zweiten Herbst-Tag- und Nachtgleiche nach der Aussaat (Schnitt 2 Wochen vor der Tag- und Nachtgleiche)	Planta: altura 2 semanas después del segundo equinoccio de otoño siguiente a la siembra (corte 2 semanas antes del equinoccio)		
QN	(a)	short	basse	niedrig	baja	Gibraltar	3
	(b)	medium	moyenne	mittel	media	Fauna	5
		tall	haute	hoch	alta	Zenith	7
15.	MG B	Plant: natural height 6 weeks after the second autumn equinox following sowing (cut 2 weeks after equinox)	Plante: hauteur naturelle six semaines après le deuxième équinoxe d'automne après le semis (coupe deux semaines après l'équinoxe)	Pflanze: Wuchshöhe 6 Wochen nach der zweiten Herbst-Tag- und Nachtgleiche nach der Aussaat (Schnitt 2 Wochen nach der Tag- und Nachtgleiche)	planta: altura 6 semanas después del segundo equinoccio de otoño siguiente a la siembra (corte 2 semanas después del equinoccio)		
QN	(a)	short	basse	niedrig	baja	Boja	3
	(b)	medium	moyenne	mittel	media	Europe	5
		tall	haute	hoch	alta	Zenith	7

	English	français	deutsch	español	Example Varieties/ Exemples/ Beispielssorten/ Variedades ejemplo	Note/ Nota
16.	MG	Plant: tendency to grow during winter	Plante: tendance à croître durant l'hiver	Pflanze: Neigung zu Wachstum im Winter	Planta: tendencia a crecer durante el invierno	
(*)	C					
(+)						
QN	Dormancy rating 1	Niveau de dormance 1	Winterruhe Stufe 1	Grado de latencia 1	Maverick	1
	Dormancy rating 2	Niveau de dormance 2	Winterruhe Stufe 2	Grado de latencia 2	Vernal	2
	Dormancy rating 3	Niveau de dormance 3	Winterruhe Stufe 3	Grado de latencia 3	Boja, Ranger	3
	Dormancy rating 4	Niveau de dormance 4	Winterruhe Stufe 4	Grado de latencia 4	Legend, Mercedes	4
	Dormancy rating 5	Niveau de dormance 5	Winterruhe Stufe 5	Grado de latencia 5	Archer	5
	Dormancy rating 6	Niveau de dormance 6	Winterruhe Stufe 6	Grado de latencia 6	Abi 700, Dorine	6
	Dormancy rating 7	Niveau de dormance 7	Winterruhe Stufe 7	Grado de latencia 7	Sutter, Oro	7
	Dormancy rating 8	Niveau de dormance 8	Winterruhe Stufe 8	Grado de latencia 8	Maricopa, Carmen	8
	Dormancy rating 9	Niveau de dormance 9	Winterruhe Stufe 9	Grado de latencia 9	CUF 101, Medina	9
	Dormancy rating 10	Niveau de dormance 10	Winterruhe Stufe 10	Grado de latencia 10	UC-1887	10
	Dormancy rating 11	Niveau de dormance 11	Winterruhe Stufe 11	Grado de latencia 11	UC-1465	11
17.	VS	Resistance to <i>Verticillium albo-atrum</i>	Résistance à <i>Verticillium albo-atrum</i>	Resistenz gegen <i>Verticillium albo-atrum</i>	Resistencia al <i>Verticillium albo-atrum</i>	
(+)	C					
QN	low	faible	gering	baja	Medalfa	3
	medium	moyenne	mittel	media	Europe, Derby	5
	high	élevée	hoch	alta	Vertus	7

	English	français	deutsch	español	Example Varieties/ Exemples/ Beispielssorten/ Variedades ejemplo	Note/ Nota
18.	VS	Resistance to	Résistance à	Resistenz gegen	Resistencia al	
(+)	C	<i>Ditylenchus dipsaci</i>	<i>Ditylenchus dipsaci</i>	<i>Ditylenchus dipsaci</i>	<i>Ditylenchus dipsaci</i>	
QN	very low	très faible	sehr gering	muy baja		1
	low	faible	gering	baja	Europe	3
	medium	moyenne	mittel	media		5
	high	élevée	hoch	alta	Vertus	7
19.	VS	Resistance to	Résistance à	Resistenz gegen	Resistencia al	
(+)	C	<i>Colletotrichum trifolii</i>	<i>Colletotrichum trifolii</i>	<i>Colletotrichum trifolii</i>	<i>Colletotrichum trifolii</i>	
QN	very low	très faible	sehr gering	muy baja	Saranac	1
	low	faible	gering	baja	Venus	3
	medium	moyenne	mittel	media		5
	high	élevée	hoch	alta	Saranac AR	7
	very high	très élevée	sehr hoch	muy alta	Arc	9
20.	VS	Resistance to	Résistance à	Resistenz gegen	Resistencia al	
(+)	C	<i>Phytophthora medicaginis</i>	<i>Phytophthora medicaginis</i>	<i>Phytophthora medicaginis</i>	<i>Phytophthora medicaginis</i>	
QN	very low	très faible	sehr gering	muy baja	Hunterfield	1
	low	faible	gering	baja		3
	medium	moyenne	mittel	media	Trifecta	5
	high	élevée	hoch	alta		7
	very high	très élevée	sehr hoch	muy alta	Aquarius	9

		English	français	deutsch	español	Example Varieties/ Exemples/ Beispielssorten/ Variedades ejemplo	Note/ Nota
21.	VS	Resistance to	Résistance à	Resistenz gegen	Resistencia al		
(+)	C	<i>Acyrtosiphon</i>	<i>Acyrtosiphon</i>	<i>kondoi</i>	<i>kondoi</i>		
QN		very low	très faible	sehr gering	muy baja	Hunter River	1
		low	faible	gering	baja		3
		medium	moyenne	mittel	media	Siriver	5
		high	élevée	hoch	alta		7
		very high	très élevée	sehr hoch	muy alta	Aurora	9
22.	VS	Resistance to	Résistance à	Resistenz gegen	Resistencia al		
(+)	C	<i>Therioaphis</i>	<i>Therioaphis</i>	<i>maculata</i>	<i>maculata</i>		
QN		very low	très faible	sehr gering	muy baja	Hunter River	1
		low	faible	gering	baja		3
		medium	moyenne	mittel	media	Trifecta	5
		high	élevée	hoch	alta		7
		very high	très élevée	sehr hoch	muy alta	Aurora	9

8. Explanations on the Table of Characteristics

8.1 *Explanations covering several characteristics*

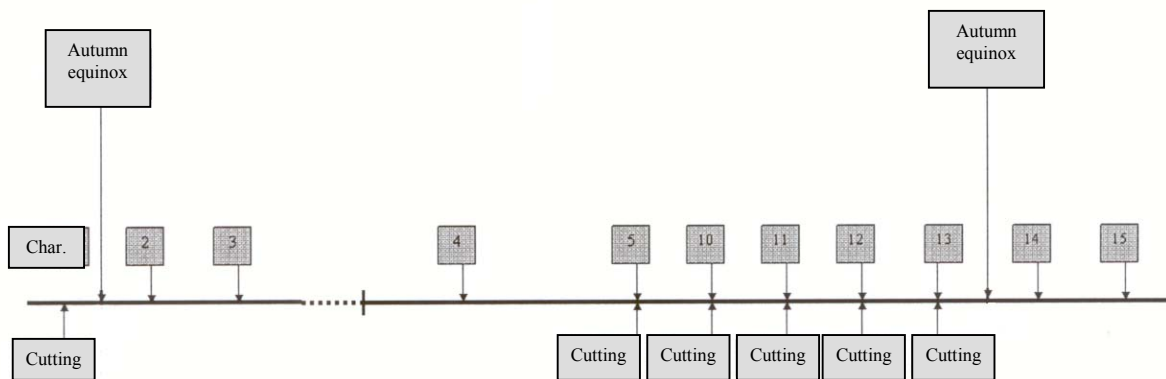
Characteristics containing the following key in the second column of the Table of Characteristics should be examined as indicated below:

(a) The equinox referred to in characteristics 2, 3, 14 and 15 and elsewhere in the text refers to the autumnal or fall equinox. This occurs on September 22 for the Northern hemisphere and March 21 for the Southern hemisphere. It is an appropriate date on which to base the plant height measurements relating to the degree of fall dormancy (conversely called “winter-activity” in many countries in the southern hemisphere).

In Characteristics 2 and 3, the plant height measurements should be respectively taken 2 and 6 weeks after the first autumnal equinox.

In Characteristics 14 and 15, 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 for each of the hemispheres.



(b) Measurements should be made on row plots on a total of 18 plants or parts of plants, 6 taken from each of the 3 replicates.

(c) Observations on flower color should be made at the beginning of flowering. The frequency should be assessed on spaced plants (see Chapter 3.3: A: spaced plants). The states of expression cover the whole range from 1% to 100%, although example varieties are so far not yet known for the whole range. Variegation is defined by the presence of yellow and violet pigments within the same flower. This combination may give the appearance of green color.

8.2 *Explanations for individual characteristics*

Ad. 4: 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. 5: Time of beginning of flowering

MS/A: 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. From the single plant data a mean date per plot and a mean date per variety are obtained.

MG/B: The date of beginning of flowering of row plots should be assessed. Row plots are considered to have headed when $\frac{1}{4}$ of inflorescence per plot can be seen. From the row plant data, a mean date per variety is obtained.

Ad. 10: Plant: natural height 3 weeks after 1st cut

The first cut should be done just after full flowering, when characteristic 9: “Stem: length of longest stem at full flowering (head included; when fully expanded)” has been assessed.

Ad. 11: Plant: natural height 3 weeks after 2nd cut

The plants should be cut just after characteristic 10 “Plant: natural height 3 weeks after 1st cut” has been measured.

Ad. 12: Plant: natural height 3 weeks after 3rd cut

The plants should be cut just after characteristic 11 “Plant: natural height 3 weeks after 2nd cut” has been measured.

Ad. 13: Plant: natural height 3 weeks after 4th cut

The plants should be cut just after characteristic 12 “Plant, natural height 3 weeks after 3rd cut” has been measured.

Ad. 16: 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 as well as the winter activity in the southern 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. 2: Plant: natural height 2 weeks after the first autumn equinox following sowing (cut 2 weeks before equinox)
- Char. 3: Plant: natural height 6 weeks after the first autumn equinox following sowing (cut 2 weeks after equinox)
- Char. 4: Plant: natural height in spring (1 month after beginning of growing the year after sowing)
- Char. 14: Plant: natural height 2 weeks after the second autumn equinox following sowing (cut 2 weeks before equinox)
- Char. 15: 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. 17: Resistance to *Verticillium albo-atrum*

- (1) The seeds are pre-germinated by sowing on wet blotting paper in Petri dishes.
- (2) 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.
- (3) 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).

Fertilizer solution for 20 liters:

(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

- (4) The plants are cut between 2 to 3 cm and are inoculated one month later.
- (5) The inoculum should be obtained after three weeks of culture made on 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.

(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 transferred to a chamber with a relative humidity of between 80 and 100%. The temperature should be 17°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:

Medalfa	low	3
Europe, Derby	medium	5
Vertus	high	7

Ad. 18: Resistance to *Ditylenchus dipsaci*

(1) Seeds are abraded, disinfected (15 minutes in Metalaxyl 1g/L) and pregerminated by sowing them in vermiculite (2000 seeds are sown to have 300 seeds germinated). It is recommended that 150 plants per variety be observed.

(2) After 4.5 days at 19°C, photoperiod of 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.

(3) The pots should be put in a climatic chamber at 19°C, 12 hours of photoperiod (11 - 15,000 lux) and 80% humidity.

(4) 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 50 nematodes between the two cotyledons and mix with carbomethylcellulose at 40%. 15 rollers per genotype are inoculated.

The humidity is set is at 100% for 4 days and reduced progressively to 80% over the 2 following days.

(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)
- stopped growth seedling (resistant seedling)
- seedling without symptoms
- dead seedling
- indeterminate 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
Vertus	high	7

Ad. 19 : Resistance 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
Medium	Potting soil mix
Temp/Light	23°C; 16+ hour day length
No. of Plants	50 per replication
No. of Reps	4 minimum
Other	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

Inoculation Procedure:

Age of Plant 7-14 days (take stand counts at 7 days)
 Type of Inoc. Spore suspension with 2 drops Tween per litre distilled water, taken from 7 day old cultures incubated at 23°C on half strength oatmeal agar.
 Concentration 2 x 10⁶ spores per ml
 Method Spray to runoff, approx. 3 ml or 5 to 10 ml per flat; place in mist chamber to maintain 100% R.H. for 48 hours at 23°C

Incubation:

Location Growth room or greenhouse at 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

Rating:

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

Check varieties (Race 1):

	Approximate Expected Resistance (%)	Acceptable Range of Resistance (%)
Resistant		
Arc	65-70	45-80
Saranac AR	45	40-60
Sequel HR	50	30-65
Susceptible		
Saranac	1	0-5
Hunter River	10	0-15

Ad. 20: Resistance to *Phytophthora medicaginis* (Hansen and Maxwell) (Phytophthora root rot)

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

Plant Culture:

Container Seedling cavities or flat within a water reservoir or a deep tub with a single drainage hole which is capable of being plugged
 Media Coarse vermiculite or a porous soil mix (e.g. 3 :2 sphagnum-based soilless mix: perlite); provide a coarse drainage layer (e.g. Gravel); pure sand medium is not desirable.

Temp/Light 20-24°C; 12-16 hr. day length
No. of Plants 50-70 per replication
No. of Reps. 3 minimum

Inoculum Culture:

Source Seedlings grown on infested soil
Storage Corn meal or V-8 juice agar.
Temperature 4-12°C
Storage life 6 months if hydrated

Inoculum Procedure:

Age of Plant 10-12 days (when first trifoliolate begins expansion)
Type of Inoc. Zoospore suspension or comminuted mycelium
Production Produce zoospores as per Miller and Maxwell (1984); or 9-day-old V-8 agar cultures of mycelium may be chopped in a blender for 10 secs
Concentration Approx. 50 zoospores or 1 ml. Chopped mycelium per seedling; mycelium prepared as: 1 culture (9 cm diam.) in 1 l water
Method For zoospores: Presaturate the soil mix and drench inoculum over the seedlings; for mycelium: drench inoculum into shallow trench and then saturate the soil with water.

Incubation:

Location Greenhouse or growth chamber
Plant counts Count at full emergence (7-8 days after seeding)
Culture Maintain flooded conditions for 2 days; keep moist until rated
Age at Rating Rate when nearly all plants of susceptible check variety are stunted and dying, i.e. for zoospores: 10-12 days after inoculation; for mycelium: 14 days after inoculation.

State of Expression	Example varieties
Highly resistant (>50%)	Aquarius
Resistant (31-50%)	
Moderately resistant (15-30%)	Trifecta
Weakly resistant (6-14%)	
Susceptible (0-<6%)	Hunterfield

Rating:

Resistant – Vigorously growing plants with only slight to no necrosis of tap and secondary roots; hypocotyl area sound with slight to no chlorosis of cotyledons.
Susceptible – Stunted or dead plants with moderate to severe necrosis of roots, hypocotyls and cotyledons.

Check varieties:

	Approximate Expected Resistance (%)	Acceptable Range of Resistance (%)
Highly Resistant		
WAPH-1	55	50-60
Aquarius	55	45-70
Resistant		
Agate	33	25-40
Susceptible		
Saranac	1	0-5
Hunterfield	4	0-7

Ad. 21 : Resistance to *Acyrtosiphon kondoi* Shinji (Blue Alfalfa Aphid)

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

Plant Culture:

Container	Flats (6 x 31 x 55 cm or similar size)
Medium	Soil mix (e.g. 8 parts sand; 3 peat; 3 perlite; 1.4% by volume of lime)
Temp/Light	22 ± 4°C; 16+ hour day length
No. of Plants	50 to 70 per replicate in rows 3 cm apart
No. of Reps	3 minimum
Other	Scarify seed and treat with fungicide to prevent damping-off; sow seed 1cm deep and cover with vermiculite

Aphid Colony:

Source	Colony consisting of blend of several field collections from area of adaptation, replenished annually
Rearing	Susceptible alfalfa in greenhouse (eg. PA-1)
Temp/Light	22 ±4°C and 16+ hour day length

Infestation Procedure:

Age of Plant	1 day after emergence; cotyledon stage; count seedlings at time of infestation
Method	Sprinkle aphids onto seedlings
Rate	Minimum of 2 aphids per seedling
Length	Approx. 21 days; spray with insecticide to terminate infestation; rate plants 7 to 10 days after spraying
Other	It is critical to maintain temperature within the range of 18 to 26°C for optimal aphid reproduction and effective resistance evaluation

Correlation to Field Reaction:

Although there have not been extensive comparisons of greenhouse and field results, it appears that levels of resistance are comparable in both situations.

Biotypes:

Although biotypes of blue alfalfa aphid are not proven to exist, there is evidence of differential reactions to resistant plants in different locations.

<u>State of Expression</u>	<u>Example varieties</u>
Highly resistant (>50%)	Aurora
Resistant (31-50%)	
Moderately resistant (15-30%)	Siriver
Weakly resistant (6-14%)	
Susceptible (0-<6%)	Hunter River

Rating:

1 Resistant	Tall, normal trifoliates
2 Resistant	Tall, small trifoliates
3 Resistant	Moderately tall, small, crinkled trifoliates
4 Susceptible	Short; small, crinkled trifoliates, usually chlorotic
5 Susceptible	Dead (= total emerged – classes 1 to 4)

Check varieties:

Values for resistant standards are totals for rating 1 to 3.

	Approximate Expected Resistance (%)	Acceptable Range of Resistance (%)
Resistant		
CUF-101	55	40-65
Aurora	60	45-75
Susceptible		
PA-1	10	5-15
Caliverde	3	0-5

Ad. 22: Resistance to *Therioaphis maculata* (Buckton) (Spotted Alfalfa Aphid)

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

Plant Culture:

Container	Flats (6 x 31 x 55 cm or similar size)
Medium	Soil mix (e.g. 8 parts sand; 3 peat; 3 perlite; 1.4% by volume of lime)
Temp/Light	26 ± 4°C; 18+ hour day length
No. of Plants	50 to 70 per replicate in rows 3 cm apart
No. of Reps	3 minimum
Other	Scarify seed and treat with fungicide to prevent damping-off; sow seed 1cm deep and cover with vermiculite

Aphid Colony:

Source Colony consisting of blend of several field collections from area of adaptation, replenished annually
Rearing Susceptible alfalfa in greenhouse (eg. Arc, Caliverde)
Temp/Light 26± 4°C and 18 hour day length

Infestation Procedure:

Age of Plant 7 to 8 days after emergence; unifoliate stage; count plants at time of infestation
Method Sprinkle aphids onto plants
Rate Minimum of 2 aphids per plant
Length Approx. 18 days or when 85% of susceptible check plants are dead and resistant check is within the expected range; spray with insecticide to terminate infestation; rate plants 10 to 15 days after spraying

Correlation to Field Reaction:

Field performance of alfalfa selected for resistance to spotted alfalfa aphid has conformed closely with expected results based on greenhouse evaluations.

Biotypes:

Performance of resistant cultivars may vary depending upon the biotype(s) present. It would be advisable to test cultivars against aphid populations in areas where they will be grown.

State of Expression

Example varieties

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

Rating:

1-2 Resistant	Plant has formed at least one trifoliate
3 Susceptible	Plant has developed very little during infestation
4 Susceptible	Plant living but has formed no trifoliates
5 Susceptible	Dead (= total emerged – classes 1 to 4)

Check varieties:

Values for resistant standards are the totals of 1-2 ratings.

	Approximate Expected Resistance (%)	Acceptable Range of Resistance (%)
Resistant		
CUF-101	60	45-75
Baker	50	35-65
Aurora	65	45-80
Susceptible		
Arc	3	0-5
Caliverde	3	0-5
Hunter River	3	0-5

9. Literature

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Gondran J., 1984: "La verticilliose de la luzerne : Détermination de l'agent causal, biologie du parasite répartition géographique, dégâts et méthode de lutte". Thèse, université des sciences de Poitiers.

Leclercq D., Caubel G., 1991: "Résistance variétale de la luzerne au nématode des tiges *Ditylenchus dipsaci* (Kühn) Filipjev ; test d'évaluation et application en sélection". Agronomie. 11, pages 603-612.

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Montegano, B., Gensollen, V., and Lassalvy S., 2002: "Fall dormancy as a descriptor of Lucerne (*Medicago sativa* L.) varieties". 19th General Meeting of the European Grassland Federation. La Rochelle, France. Pages 452-453.

Roulier. G., Guy P., 1986: "Stades phénologiques de la luzerne, outil pour l'éleveur". Le Sélectionneur Français. 37, pages 85-90.

Teuber, L.R., Taggard, K.L., Gibbs, L.K., Mccaslin, M.H., Peterson, M.A., Barnes, D.K., 1998: "Fall Dormancy. In Standard tests to characterize alfalfa cultivars". 3rd ed. (amended 1998). North American Alfalfa Improvement Conference, Beltsville, MD. (Available on line at <http://www.naaic.org/stdtests/Dormancy2.html>) (Verified July 11, 2003).

U.S. Department of Agriculture, Agricultural Research Service: "A System for Visually Classifying Alfalfa Flower Color". Agriculture Handbook No. 424. (Available on line at <http://www.naaic.org/Resources/colorguide/flowercolor.html>).

10. Technical Questionnaire

TECHNICAL QUESTIONNAIRE	Page {x} of {y}	Reference Number:
		Application date: (not to be filled in by the applicant)
TECHNICAL QUESTIONNAIRE to be completed in connection with an application for plant breeders' rights		
1.1.1 Botanical name	<input type="text" value="Medicago sativa L."/>	
1.1.2 Common name	<input type="text" value="Lucerne, Alfalfa"/>	[]
1.2.1 Botanical name	<input type="text" value="Medicago x varia Martyn"/>	
1.2.2 Common name	<input type="text" value="Hybrid Lucerne"/>	[]
2. Applicant		
Name	<input type="text"/>	
Address	<input type="text"/>	
Telephone No.	<input type="text"/>	
Fax No.	<input type="text"/>	
E-mail address	<input type="text"/>	
Breeder (if different from applicant)	<input type="text"/>	

TECHNICAL QUESTIONNAIRE	Page {x} of {y}	Reference Number:
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3. Proposed denomination and breeder's reference

Proposed denomination
(if available)

Breeder's reference

#4. Information on the breeding scheme and propagation of the variety

4.1 Breeding scheme

Variety resulting from:

4.1.1 Crossing

- (a) controlled cross []
(please state parent varieties)
- (b) partially known cross []
(please state known parent variety(ies))
- (c) totally unknown cross []

4.1.2 Mutation []
(please state parent variety)

4.1.3 Discovery []
(please state where, when and how developed)

4.1.4 Other []
(please provide details)

4.2 Method of propagating the variety

Authorities may allow certain of this information to be provided in a confidential section of the Technical Questionnaire.

TECHNICAL QUESTIONNAIRE	Page {x} of {y}	Reference Number:
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5. Characteristics of the variety to be indicated (the number in brackets refers to the corresponding characteristic in Test Guidelines; please mark the note which best corresponds).

Characteristics	Example Varieties	Note
5.1 (6)	Flower: frequency of plants with very dark blue violet flowers	
absent or very low	Diane	1[]
low	Sanditi	3[]
medium	Andela	5[]
high	Orca	7[]
5.2 (7)	Flower: frequency of plants with variegated flowers	
absent or very low	Symphonie	1[]
low	Luzelle, Letizia	3[]
medium	Franken Neu, Karlu (M.v)	5[]
high		7[]
5.3 (8)	Flower: frequency of plants with cream, white or yellow flowers	
absent or very low	Europe	1[]
low		3[]
medium	Karlu (M.v)	5[]
high		7[]

TECHNICAL QUESTIONNAIRE	Page {x} of {y}	Reference Number:
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Characteristics	Example Varieties	Note
5.4 Plant: tendency to grow during winter (16)		
Dormancy rating 1	Maverick	1[]
Dormancy rating 2	Vernal	2[]
Dormancy rating 3	Boja, Ranger	3[]
Dormancy rating 4	Legend, Mercedes	4[]
Dormancy rating 5	Archer	5[]
Dormancy rating 6	Abi 700, Dorine	6[]
Dormancy rating 7	Sutter, Oro	7[]
Dormancy rating 8	Maricopa, Carmen	8[]
Dormancy rating 9	CUF 101, Medina	9[]
Dormancy rating 10	UC-1887	10[]
Dormancy rating 11	UC-1465	11[]

6. Similar varieties and differences from these varieties

Please use the following table and box for comments to provide information on how your candidate variety differs from the variety (or varieties) which, to the best of your knowledge, is (or are) most similar. This information may help the examination authority to conduct its examination of distinctness in a more efficient way.

Denomination(s) of variety(ies) similar to your candidate variety	Characteristic(s) in which your candidate variety differs from the similar variety(ies)	Describe the expression of the characteristic(s) for the similar variety(ies)	Describe the expression of the characteristic(s) for your candidate variety
<i>Example</i>	<i>Plant: tendency to grow during winter</i>	<i>Dormancy rating 3</i>	<i>Dormancy rating 4</i>

Comments:

TECHNICAL QUESTIONNAIRE	Page {x} of {y}	Reference Number:
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#7. Additional information which may help in the examination of the variety

7.1 In addition to the information provided in sections 5 and 6, are there any additional characteristics which may help to distinguish the variety?

Yes [] No []

(If yes, please provide details)

7.2 Are there any special conditions for growing the variety or conducting the examination?

Yes [] No []

(If yes, please provide details)

7.3 Other information

8. Authorization for release

(a) Does the variety require prior authorization for release under legislation concerning the protection of the environment, human and animal health?

Yes [] No []

(b) Has such authorization been obtained?

Yes [] No []

If the answer to (b) is yes, please attach a copy of the authorization.

Authorities may allow certain of this information to be provided in a confidential section of the Technical Questionnaire.

TECHNICAL QUESTIONNAIRE	Page {x} of {y}	Reference Number:
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9. Information on plant material to be examined or submitted for examination

9.1 The expression of a characteristic or several characteristics of a variety may be affected by factors, such as pests and disease, chemical treatment (e.g. growth retardants or pesticides), effects of tissue culture, different rootstocks, scions taken from different growth phases of a tree, etc.

9.2 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 the plant material has undergone such treatment, full details of the treatment must be given. In this respect, please indicate below, to the best of your knowledge, if the plant material to be examined has been subjected to:

- | | | |
|---|---------|--------|
| (a) Microorganisms (e.g. virus, bacteria, phytoplasma) | Yes [] | No [] |
| (b) Chemical treatment (e.g. growth retardant, pesticide) | Yes [] | No [] |
| (c) Tissue culture | Yes [] | No [] |
| (d) Other factors | Yes [] | No [] |

Please provide details of where you have indicated "yes".

.....

10. I hereby declare that, to the best of my knowledge, the information provided in this form is correct:

Applicant's name

Signature

Date

[End of document]