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UPOV

INTERNATIONAL UNION FOR THE PROTECTION OF NEW VARIETIES OF PLANTS

GENEVA

TECHNICAL WORKING PARTY FOR AGRICULTURAL CROPS

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DRAFT TEST GUIDELINES FOR WHITE CLOVER
DOCUMENT TG/38/7(PROJ.1)

Document prepared by experts from the United Kingdom

The attached document TG/38/7(proj.1) already incorporates the standard wording of document TGP/7.2, which was adopted by the Technical Committee at its thirty-eighth session in April 2002, and includes some additional standard wording from document TGP/7.1 Draft 1, also agreed at that session.

[Document TG/38/7(proj.1) follows]

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ORIGINAL: English
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INTERNATIONAL UNION FOR THE PROTECTION OF NEW VARIETIES OF PLANTS

GENEVA

WHITE CLOVER*

*(Trifolium repens L.)**

GUIDELINES

FOR THE CONDUCT OF TESTS

FOR DISTINCTNESS, UNIFORMITY AND STABILITY

Alternative Names:*

Latin	English	French	German	Spanish
Trifolium repens L.	White clover	Trèfle blanc	Weissklee	Trébol blanco

ASSOCIATED DOCUMENTS

These guidelines should be read in conjunction with document TG/1/3, "General Introduction to the Examination of Distinctness, Uniformity and Stability and the Development of Harmonized Descriptions of New Varieties of Plants" (hereinafter referred to as 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 Guidelines

1.1 These Test Guidelines apply to all varieties of *Trifolium repens* L.

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:

1.0 kg.

2.4 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.

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

2.6 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 Duration of Tests

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

3.2 Testing Place

The tests should normally be conducted at one place. If any characteristics of the variety, which are relevant for the examination of DUS, cannot be seen at that place, the variety may be tested at an additional place.

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.

3.3.1 Type of observation – visual or measurement

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

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

3.3.2 Type of plot for observation

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 plant
B: row plot
C: special test

3.4 Test Design

3.4.1 General

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.

3.4.2 Plot design

Each test should be designed to result in a total of, at least 60 spaced plants and 10 meters of row plot.

Plots with single spaced plants: Each test should consist of 60 single spaced plants per variety arranged in 3, 4, 5 or 6 replicates, i.e. plots of 20, 15, 12 or 10 plants

Row plots: Each test which includes row plots should consist of at least 10 meters of row arranged in two replicates, each of 5 meters. The density of sowing should be such that about 200 plants per meter should be obtained.

3.5 Number of Plants / Parts of Plants to be Examined

Unless otherwise indicated, all observations determined by measuring or counting should be made on 60 plants or parts taken from each of 60 plants.

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

4.1.1.1 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.

4.1.1.2 Characteristics should be measured so that a mean value per plot can be obtained: from these data a standard deviation per variety can be derived and the data submitted to a 'two-way' analysis of variance. The significance of measured differences should be taken into account for assessing distinctness and the preparation of descriptions.

4.1.2 Consistent Differences

The minimum duration of tests recommended in section 3.1 reflects, in general, the need to ensure that any differences in a characteristic are sufficiently consistent.

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 for cross-pollinated varieties should be according to the recommendations in the General Introduction.

4.2.3 For the assessment of uniformity of a variety, the standard deviation of the mean value for each characteristic should be compared with the mean of the standard deviations of comparable varieties using a recognized statistical technique.

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 or plant 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 is 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) Leaf: size of median leaflet (characteristic 13)

(b) Leaf: Intensity of white marks (characteristic 4)

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.

6.5 Legend

(*) Asterisked characteristic – see Section 6.1.2

(QL) Qualitative characteristic – see Section 6.3
(QN) Quantitative characteristic – see Section 6.3
(PQ) Pseudo-Qualitative characteristic – see Section 6.3

(+) See Explanations on the Table of Characteristics in Chapter 8.

MS, VG, VS } Type of observation – see Section 3.3.1

A, B, C } Type of plot for observation – see Section 3.3.2

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

Char. No.	Method of Examination	English	français	deutsch	español	Example Varieties Exemples Beispielssorten Variedades ejemplo	Note/ Nota
(1)	**A**	**Plant: tendency to form inflorescences without vernalization**	**Plante: tendance à former des inflorescences**	**Pflanze: Neigung zur Bildung von Blütenständen**	**Planta: tendencia a formar inflorescencias**		
(+)	**VS**						
		absent or very weak	nulle ou très faible	fehlend oder sehr gering	ausente o muy débil	Barbian	1
		weak	faible	gering	débil	Aran	3
		medium	moyenne	mittel	media	Milkanova	5
		strong	forte	stark	fuerte	Lune de Mai	7
		very strong	très forte	sehr stark	muy fuerte	Tivoli	9
2.	**A**	**Plant: intensity of green color**	**Plante: intensité de la couleur verte**	**Pflanze: Intensität der Grünfärbung**	**Planta: intensidad del color verde**		
(+)	**VS**						
		light	claire	hell	claro	Avoca	3
		medium	moyenne	mittel	medio	Milkanova	5
		dark	foncée	dunkel	obscuro	Brindisi	7
3.	**C**	**Plant: proportion of plants with cyanid glucoside**	**Plante: proportion de plantes à glucosides cyanogènes**	**Pflanze: Anteil Pflanzen mit Cyanglukosid**	**Planta: proporción de plantas con glucosidos cianogenéticos**		
(+)							
		absent or very low	absente ou très faible	fehlend oder sehr gering	ausente o muy baja	Pertina	1
		low	faible	gering	baja	Barbian	3
		medium	moyenne	mittel	media	Grasslands Tahora	5
		high	élevée	stark	alta	Avoca	7
		very high	très élevée	sehr stark	muy alta	Grasslands Pitau	9

Char. No.	Method of Examination	English	français	deutsch	español	Example Varieties Exemples Beispielssorten Variedades ejemplo	Note/ Nota
4. (*) (+)	**A VS**	**Plant: intensity of white leaf marks**	**Planté: intensité des marques foliaires blanches**	**Pflanze: Intensität der weißen Blattzeichnung**	**Planta: intensidad de las marcas foliares blancas**		
		absent or very weak	nulle ou très faible	fehlend oder sehr gering	ausente o muy débil	Steinacher Weißklee	1
		weak	faible	gering	débil		3
		medium	moyenne	mittel	media	Asterix	5
		strong	forte	stark	fuerte		7
		very strong	très forte	sehr stark	muy fuerte	Haifa	9
5. (*) (+)	**A MS**	**Plant: time of flowering**	**Plante: époque de floraison**	**Pflanze: Zeitpunkt der Blüte**	**Planta: época de la floración**		
		very early	très précoce	sehr früh	muy precoz	Haifa	1
		early	précoce	früh	precoz	Chieftain	3
		medium	moyenne	mittel	media	Grasslands Huia	5
		late	tardive	spät	tardía	Tivoli	7
		very late	très tardive	sehr spät	muy tardía	Regal	9
6.	**A MS**	**Plant: natural height**	**Plante: hauteur naturelle**	**Pflanze: natürliche Höhe**	**Planta: altura**		
		short	courte	niedrig	corta	Kent Wild White	3
		medium	moyenne	mittel	media	Pertina	5
		tall	longue	hoch	larga	Milkanova	7
7.	**A MS**	**Plant: width**	**Plante: largeur**	**Pflanze: Breite**	**Planta: anchura**		
		narrow	étroite	schmal	estrecha	Asterix	3
		medium	moyenne	mittel	media	Regal	5
		broad	large	breit	ancha	Aran	7

Char. No.	Method of Examination	English	français	deutsch	español	Example Varieties Exemples Beispielssorten Variedades ejemplo	Note/ Nota
8. **(+)**	**A** **MS**	**Stem: thickness of stolon**	**Tige: grosseur du stolon**	**Stengel: Ausläuferdicke**	**Tallo: grosor del estolón**		
		very thin	très fin	sehr dünn	muy delgado	Kent Wild White	1
		thin	fin	dünn	delgado	Barbian	3
		medium	moyen	mittel	medio	Grasslands Huia	5
		thick	gros	dick	grueso	Kersey	7
		very thick	très gros	sehr dick	muy grueso	Aran	9
9. **(+)**	**A** **MS**	**Leaf: thickness of petiole**	**Feuille: grosseur du pétiole**	**Blatt: Dicke des Blattstiels**	**Hoja: grosor del pecíolo**		
		very thin	très fin	sehr dünn	muy delgado	Kent Wild White	1
		thin	fin	dünn	delgado	Barbian	3
		medium	moyen	mittel	medio	Avoca	5
		thick	gros	dick	grueso	Milkanova	7
		very thick	très gros	sehr dick	muy grueso	Regal	9
10. **(+)**	**A** **MS**	**Leaf: length of petiole**	**Feuille: longueur du pétiole**	**Blatt: Länge des Blattstiels**	**Hoja: longitud del pecíolo**		
		short	courte	kurz	corta	Asterix	3
		medium	moyenne	mittel	media	Grasslands Huia	5
		long	longue	lang	larga	Chieftain	7
11. **(*)** **(+)**	**A** **MS**	**Leaf: length of median leaflet**	**Feuille: longueur de la foliole médiane**	**Blatt: Länge des mittleren Fiederblatts**	**Hoja: longitud del folíolo central**		
		very short	très courte	sehr kurz	muy corta	Kent Wild White	1
		short	courte	kurz	corta	Barbian	3
		medium	moyenne	mittel	media	Avoca	5
		long	longue	lang	larga	Grasslands Pitau	7
		very long	très longue	sehr lang	muy larga	Aran	9

Char. No.	Method of Examination	English	français	deutsch	español	Example Varieties Exemples Beispielssorten Variedades ejemplo	Note/ Nota
12. (*) (+)	**A MS**	**Leaf: width of median leaflet**	**Feuille: largeur de la foliole médiane**	**Blatt: Breite des mittleren Fieder-blatts**	**Hoja: anchura del folíolo central**		
		very narrow	très étroite	sehr schmal	muy estrecha	Kent Wild White	1
		narrow	étroite	schmal	estrecha	Barbian	3
		medium	moyenne	mittel	media	Grasslands Huia	5
		broad	large	breit	ancha	Grasslands Pitau	7
		very broad	très large	sehr breit	muy ancha	Aran	9
13 (*) (+)	**A MS**	**Leaf: size of median leaflet**	**Feuille: taille de la foliole médiane**	**Blatt: Große des mittleren Fieder-blatts**	**Hoja: tamaño del folíolo central**		
		very small	très petit	sehr klein	muy pequeño	Kent Wild White	1
		small	petit	klein	pequeño	Rivendel	3
		medium	moyen	mittel	medio	Pertina	5
		large	grand	groß	grande	Grasslands Pitau	7
		very large	très grand	sehr groß	muy grande	Aran	9
14. (*) (+)	**A MS**	**Leaf: ratio of length to width of median leaflet**	**Feuille: rapport longueur/ largeur de la foliole latérale**	**Blatt: Verhältnis Länge/Breite des mittleren Fiederblatts**	**Hoja: relación longitud/ anchura del folíolo central**		
		small	petit	klein	pequeño	Donna	3
		medium	moyen	mittel	medio	Barbian	5
		large	grand	groß	grande	Rivendel	7
15.	**A MS**	**Flower: length of peduncle**	**Fleur: longueur du pédoncule**	**Blute: Länge des Blütenstands-stiels**	**Flor: longitud del pedúnculo**		
		short	court	kurz	corto	Kent Wild White	3
		medium	moyen	mittel	medio	Grasslands Huia	5
		long	long	lang	alto	Aran	7

Char. No.	Method of Examination	English	français	deutsch	español	Example Varieties Exemples Beispielssorten Variedades ejemplo	Note/ Nota
16.	**A** **VS**	**Plant: number of flower heads**	**Plante: nombre d'inflorescences**	**Pflanze: Anzahl Blütenstände**	**Planta: número de cabezas florales**		
		small	petit	klein	pequeño	Regal	3
		medium	moyen	mittel	medio	Avoca	5
		large	grand	groß	grande	Milkanova	7

8. Explanations on the Table of Characteristics

Ad. 1: Plant: tendency to form inflorescences without vernalization

The observation should be made before the period of verbalization. The number of flower heads produced on each plant should be assessed and scored.

Ad. 2: Plant: intensity of green color

The observation should be made in the vegetative phase by examination and scoring the overall green color of the plant.

Ad. 3: Plant: proportion of plants with cyanid glucoside

GUIGNARD Test

Preparation of picro-sodic paper

1. A hot aqueous solution of 1% picric acid is prepared, to which 10% of sodium carbonate is added after cooling down.

2. Strips of Whatmann No.1 filter paper are dipped into the solution and then kept slightly humid, protected against light and heat.

Test Procedure

1. Healthy leaves are selected from each of sixty plants, mashed individually and put into separate test tubes.

2. A drop of toluol is added to each test tube and a strip of picro-sodic paper is secured by the stopper, with the base of the paper kept approximately 5mm above the leaf material.

3. The tubes are placed in darkness in a water-bath at 27-30°C for 4 hours.

4. If there is HCN present the paper will change color from yellow to red. This color reaction is observed and recorded for each of the 60 plants.

Ad. 4: Plant: intensity of white leaf marks

The observation should be made at beginning of flowering by examination and scoring of the plant as a whole. The presence of any type of white mark or the complete absence of marks is recorded.

Ad. 5: Plant: time of flowering

A plant is recorded as flowering when three florets on three separate flower heads are showing color. The observation should be made at least twice weekly.

Ad. 8: Stem: thickness of stolon

After all the plants of each variety in a replicate have flowered, and within 1–2 weeks after flowering, the longest healthily growing stolon should be selected from each plant for measurement.

The thickness (diameter) of the stolon should be measured at a point midway between the third and the fourth internode counted from the growing tip.

Ad. 9, 10: Leaf: thickness (9), and length (10),of petiole

Time of measurement and selection of stolon as for characteristic 8.

The petiole of the third expanded leaf of the stolon, counted from the growing tip, should be measured at its widest point.

Ad. 11, 12: Leaf: length (11), width (12), of median leaflet

Time of measurement and selection of stolon as for characteristics 8, 9 and 10.

The median leaflet of the third expanded leaf from the growing tip of the stolon should be selected for measurement of its length and width.

Ad. 13: Leaf: size of median leaflet

Calculated from the product of leaf: length (10) x leaf width (12).

Ad. 14: Leaf: ratio of length to width of median leaflet

Calculated from the ratio of leaf: length (10) ÷ leaf width (12).

Ad. 15: Flower: length of peduncle

A mature flower taken from close to center of the plant is selected for measurement of its peduncle length.

Ad. 16: Plant: number of flower heads

The number of flower heads per plant is assessed on each of the 60 plants of a variety at maturity, normally 30 days after the mean date of flowering of the variety.

9. Literature

10. Technical Questionnaire

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	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. Subject of the Technical Questionnaire

1.1 *Latin Name*	*Trifolium repens* L.
1.2 Common Name	WHITE CLOVER

2. Applicant

Name	
Address	
Telephone No.	
Fax No.	
E-mail address	

Breeder (if different from applicant)

3. Proposed denomination and breeder's reference

Proposed denomination (if available)	
Breeder's reference	

TECHNICAL QUESTIONNAIRE	Page 2 of 6	Reference Number:

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

4.1 Breeding Scheme

4.2 Method of Propagating the Variety

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 (4)	**Plant: intensity of white leaf marks**		
	absent or very weak	Steinacher, Weißklee	1 [ ]
	weak		3 [ ]
	medium	Asterix	5 [ ]
	strong		7 [ ]
	very strong	Haifa	9 [ ]

TECHNICAL QUESTIONNAIRE	Page 3 of 6	Reference Number:

	Characteristics	Example Varieties	Note
5.2 (5)	**Plant: time of flowering**		
	very early	Haifa	1 [ ]
	early	Chieftain	3 [ ]
	medium	Grasslands Huia	5 [ ]
	late	Tivoli	7 [ ]
	very late	Regal	9 [ ]
5.3 (11)	**Leaf: length of median leaflet**		
	very short	Kent Wild White	1 [ ]
	short	Barbian	3 [ ]
	medium	Avoca	5 [ ]
	long	Grasslands Pitau	7 [ ]
	very long	Aran	9 [ ]
5.4 (12)	**Leaf: width of median leaflet**		
	very narrow	Kent Wild White	1 [ ]
	narrow	Barbian	3 [ ]
	medium	Grasslands Huia	5 [ ]
	broad	Grasslands Pitau	7 [ ]
	very broad	Aran	9 [ ]
5.5 (13)	**Leaf: size of median leaflet**		
	very small	Kent Wild White	1 [ ]
	small	Rivendel	3 [ ]
	medium	Pertina	5 [ ]
	large	Grasslands Pitau	7 [ ]
	very large	Aran	9 [ ]

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6. Similar varieties and differences from these varieties

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
(Example)	*Plant: height*	*e.g. note 3*	*note 7*
		e.g. short	*tall*
		e.g. 90 cm	*130 cm*

TECHNICAL QUESTIONNAIRE	Page 5 of 6	Reference Number:

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?

7.1.1 Resistance to pest and diseases

Yes [ ] No [ ]

(If yes, please provide details)

7.1.2 Other

Yes [ ] No [ ]

(If yes, please provide details)

7.2 Special conditions for the examination of the variety

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

Yes [ ] No [ ]

7.2.2 If yes, please give 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.

TECHNICAL QUESTIONNAIRE	Page 6 of 6	Reference Number:

9. 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]