



**TWA/30/4**

**ORIGINAL:** English

**DATE:** July 2, 2001

**INTERNATIONAL UNION FOR THE PROTECTION OF NEW VARIETIES OF PLANTS**

GENEVA

**TECHNICAL WORKING PARTY  
FOR  
AGRICULTURAL CROPS**

**Thirtieth Session**

**Texcoco, Mexico, September 3 to 7, 2001**

WORKING PAPER ON REVISED TEST GUIDELINES FOR WHITE CLOVER

*Document prepared by the experts from the United Kingdom*

TABLE OF CONTENTS

I.	Subject of these Guidelines .....	3
II.	Material Required .....	3
III.	Conduct of Tests .....	3
IV.	Methods and Observations.....	4
V.	Grouping of Varieties .....	4
VI.	Characteristics and Symbols .....	4
VII.	Table of Characteristics .....	6
VIII.	Explanations on the Table of Characteristics .....	11
IX.	Literature.....	13
X.	Technical Questionnaire.....	14

## I. Subject of these Guidelines

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

## II. Material Required

1. The competent authorities decide when, where and in what quantity and quality the plant material required for testing the variety is to be delivered. Applicants submitting material from a State other than that in which the testing takes place must make sure that all customs formalities are complied with. The minimum quantity of seed to be supplied by the applicant in one or several samples should be:

1.0 kg.

The seed should at least meet the minimum requirements for germination capacity, moisture content and purity for marketing certified seed in the country in which the application is made. The germination capacity should be as high as possible.

2. The plant material must not have undergone any treatment unless the competent authorities allow or request such treatment. If it has been treated, full details of the treatment must be given.

## III. Conduct of Tests

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

2. The tests should normally be conducted at one place. If any important characteristics of the variety cannot be seen at that place, the variety may be tested at an additional place.

3. The field tests should be carried out under conditions ensuring normal growth. The size of the plots should be such that plants or parts of plants may be removed for measurement and counting without prejudice to the observations which must be made up to the end of the growing period. Each test at each testing place should include, per growing period, 60 spaced plants and may in addition include 10 metres of row.

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. More replicates are generally more efficient when fewer varieties are included in the test.

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

Separate plots for observation and for measuring can only be used if they have been subject to similar environmental conditions.

4. Additional tests for special purposes may be established.

#### IV. Methods and Observations

1. Unless otherwise stated, all measurements for assessment of distinctness, uniformity and stability should be made on 60 plants or parts taken from each of 60 plants.
2. Characteristics should be measured on each plant in the trial 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.
3. For the assessment of distinctness of a variety, the mean values for each characteristic should be compared with those for other varieties using a recognized statistical technique. (See TGP/8; TGP/9)
4. For the assessment of uniformity of a variety and the inference of its stability, 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. (See TGP/8; TGP/10; TGP/11)

#### V. Grouping of Varieties

1. The collection of varieties to be grown should be divided into groups to facilitate the assessment of distinctness. Characteristics which are suitable for grouping purposes are those which are known from experience not to vary, or to vary only slightly, within a variety. Their various states of expression should be fairly evenly distributed throughout the collection.
2. It is recommended that the competent authorities use the following characteristics for grouping varieties within each subspecies:
  - (a) Leaf: size of median leaflet (characteristic 13)

#### VI. Characteristics and Symbols

1. To assess distinctness, uniformity and stability, the characteristics and their states as given in the Table of Characteristics should be used.
2. Notes (numbers), for the purposes of electronic data processing, are given opposite the states of expression for each characteristic.

#### 3. Legend

(\*) Characteristics that should be used on all varieties in every growing period over which examinations are made and always be included in the variety descriptions, except when the state of expression of a preceding characteristic or regional environmental conditions render this impossible.

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

1) To be observed on: A = spaced plants  
B = row plots  
C = special test

M = actual measurement

C = calculated by mathematical combination of two measured characteristics

VG = visual assessment by a single observation of a group of plants or parts of plants

VS = visual assessment by observation of a number of individual plants or parts of plants

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

Plot <sup>1)</sup> Parcelle <sup>1)</sup> English Parzelle <sup>1)</sup> Parcela <sup>1)</sup>		français	deutsch	español	Example Varieties Exemples Beispielssorten Variedades ejemplo	Note/ Nota
(1) A VS (+)	<b>Plant: tendency to form inflorescences in year of sowing</b>	<b>Plante : tendance à former des inflorescences l'année du semis</b>	<b>Pflanze: Neigung zur bildung von Blütenständen im Ausaatjahr</b>	<b>Planta: tendencia a formar inflorescencias el año de siembra</b>		
	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 VS (+)	<b>Plant: intensity of green color</b>	<b>Plante: intensité de la couleur verte</b>	<b>Pflanze: Intensität der Grünfärbung</b>	<b>Planta: intensidad del color verde</b>		
	light	claire	hell	claro	Avoca	3
	medium	moyen	mittel	medio	Milkanova	5
	dark	foncée	dunkel	oscuro	Brindisi	7
3. C (+)	<b>Plants: proportion with cyanid glucoside</b>	<b>Plantes: proportion à glucosides cyanogènes</b>	<b>Pflanzen: Anteil mit Cianglukosid</b>	<b>Plantas: proporción de glucosido cianogenético</b>		
	absent or very low	absente ou très faible	fehlent 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

Plot <sup>1)</sup> Parcelle <sup>1)</sup> Parzelle <sup>1)</sup> Parcela <sup>1)</sup>		English	français	deutsch	español	Example Varieties Exemples Beispielssorten Variedades ejemplo	Note/ Nota
<b>4.</b> (*) (+)	<b>A</b> <b>VS</b>	<b>Leaf: intensity of white marks</b>	<b>Feuille: intensité des marques foliaires blanches</b>	<b>Blatt: Intensität der weißen Zeichnung</b>	<b>Hoja: intensidad de las marcas blancas</b>		
		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
<b>5.</b> (*) (+)	<b>A</b> <b>M</b>	<b>Plant: time of flowering</b>	<b>Planté : époque de floraison</b>	<b>Pflanze: Zeitpunkt der Blüte</b>	<b>Planta: época de la floración</b>		
		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
<b>6.</b>	<b>A</b> <b>VG</b>	<b>Plant: natural height</b>	<b>Plante: hauteur naturelle</b>	<b>Pflanze: natürliche Höhe</b>	<b>Planta: altura natural</b>		
		short	courte	niedrig	corta	Kent Wild White	3
		medium	moyenne	mittel	media	Pertina	5
		tall	longue	hoch	larga	Milkanova	7
<b>7.</b>	<b>A</b> <b>VG</b>	<b>Plant: width</b>	<b>Plante: largeur</b>	<b>Pflanze: Breite</b>	<b>Planta: anchura</b>		
		narrow	petite	schmal	estrecha	Asterix	3
		medium	moyenne	mittel	media	Regal	5
		broad	grande	breit	ancha	Aran	7

	Plot <sup>1)</sup> Parcelle <sup>1)</sup> Parzelle <sup>1)</sup> Parcela <sup>1)</sup>	English	français	deutsch	español	Example Varieties Exemples Beispielssorten Variedades ejemplo	Note/ Nota
<b>8.</b>	<b>A</b>	<b>Stem: thickness of stolon</b>	<b>Tige: grosseur du stolon</b>	<b>Stengel: Ausläufer-dicke</b>	<b>Tallo: grosor del estolón</b>		
(+)	<b>VG</b>						
		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
<b>9.</b>	<b>A</b>	<b>Leaf: thickness of petiole</b>	<b>Feuille: grosseur du pétiole</b>	<b>Blatt: Dicke des Blattstiels</b>	<b>Hoja: grosor del pecíolo</b>		
(+)	<b>VG</b>						
		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
<b>10.</b>	<b>A</b>	<b>Leaf: length of petiole</b>	<b>Feuille: longueur du pétiole</b>	<b>Blatt: Länge des Blattstiels</b>	<b>Hoja: longitud del pecíolo</b>		
(+)	<b>VG</b>						
		short	courte	kurz	corta	Asterix	3
		medium	moyenne	mittel	media	Grasslands Huia	5
		long	longue	lang	larga	Chieftain	7
<b>11.</b>	<b>A</b>	<b>Leaf: length of median leaflet</b>	<b>Feuille: longueur de la foliole médiane</b>	<b>Blatt: Länge des mittleren Fiederblatts</b>	<b>Hoja: longitud del folíolo central</b>		
(*)	<b>VG</b>						
(+)							
		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



Plot <sup>1)</sup> Parcelle <sup>1)</sup> Parzelle <sup>1)</sup> Parcela <sup>1)</sup>	English	français	deutsch	español	Example Varieties Exemples Beispielssorten Variedades ejemplo	Note/ Nota
<b>12. A</b> <b>(*)</b> <b>(+)</b> <b>VG</b>	<b>Leaf: width of median leaflet</b>	<b>Feuille: largeur de la foliole médiane</b>	<b>Blatt: Breite des mittleren Fiederblatts</b>	<b>Hoja: anchura del folíolo central</b>		
	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
<b>13. A</b> <b>(*)</b> <b>(+)</b> <b>C</b>	<b>Leaf: size of median leaflet</b>	<b>Feuille: taille de la foliole médiane</b>	<b>Blatt: Größe des mittleren Fiederblatts</b>	<b>Hoja: tamaño del folíolo central</b>		
	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	gros	grande	Grasslands Pitau	7
	very large	très grand	sehr gros	muy grande	Aran	9
<b>14. A</b> <b>(*)</b> <b>(+)</b> <b>C</b>	<b>Leaf: shape of median leaflet</b>	<b>Feuille: forme de la foliole latérale</b>	<b>Blatt: Form des mittleren Fiederblatts</b>	<b>Hoja: forma del folíolo central</b>		
	elongated	allongée	länglich	alargada	Donna	1
	ovate	ovale	eiförmig	ovalada	Barbian	2
	rounded	arrondie	abgerundet	redondeada	Rivendel	3
<b>15. A</b> <b>VG</b>	<b>Flower: length of peduncle</b>	<b>Fleur: longueur du pédoncule</b>	<b>Blute: Länge des Blütenstandsstiele</b>	<b>Flor: longitud del pedúnculo</b>		
	short	court	kurz	corto	Kent Wild White	3
	medium	moyen	mittel	medio	Grasslands Huia	5
	long	long	lang	alto	Aran	7

Plot <sup>1)</sup> Parcelle <sup>1)</sup> English Parzelle <sup>1)</sup> Parcela <sup>1)</sup>		français	deutsch	español	Example Varieties Exemples Beispielssorten Variedades ejemplo	Note/ Nota
<b>16. A</b>	<b>Plant: number of flower heads</b>	<b>Plante: nombre d'inflorescences</b>	<b>Pflanze: Anzahl Blütenstände</b>	<b>Planta: número de cabezas florales</b>		
<b>VS</b>	small	petit	klein	pequeño	Regal	5
	medium	moyen	mittel	medio	Avoca	7
	large	grand	gros	grande	Milkanova	9

### VIII. Explanations on the Table of Characteristics

#### Ad. 1: Plant: tendency to form inflorescences in year of sowing [PROPOSED]

The observation should be made in the autumn of the year of sowing . 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 autumn of the year of sowing by examination and scoring the overall green color of the plant.

#### Ad. 3: Plants: proportion 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: Leaf: intensity of white 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 from the growing tip of the stolon should be selected for measurement of its length and width.

Ad. 13: Leaf: size of median leaflet [PROPOSED]

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

Ad. 14: Leaf: shape of median leaflet [PROPOSED]

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

Ad. 15: Flower: length of peduncle [PROPOSED]

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 [PROPOSED]

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.

IX. Literature

X. Technical Questionnaire

Reference Number  
(not to be filled in by the applicant)

TECHNICAL QUESTIONNAIRE  
to be completed in connection with an application for plant breeders' rights

1. Species *Trifolium repens* L.  
WHITE CLOVER

2. Applicant (Name and address)

3. Proposed denomination or breeder's reference

4. Information on origin, maintenance and reproduction of the variety (number of components and generation, origin etc.)

5. Characteristics of the variety to be indicated (the number in brackets refers to the corresponding characteristic in Test Guidelines; please mark the state of expression which best corresponds).

Characteristics	Example Varieties	Note
<b>5.1 Leaf: intensity of white marks (4)</b>		
absent or very weak	Steinacher, Weißklee	1 [ ]
weak		3 [ ]
medium	Asterix	5 [ ]
strong		7 [ ]
very strong	Haifa	9 [ ]
<b>5.2 Plant: time of flowering (5)</b>		
very early	Haifa	1 [ ]
early	Chieftain	3 [ ]
medium	Grasslands Huia	5 [ ]
late	Tivoli	7 [ ]
very late	Regal	9 [ ]
<b>5.3 Leaf: length of median leaflet (11)</b>		
very short	Kent Wild White	1 [ ]
short	Barbian	3 [ ]
medium	Avoca	5 [ ]
long	Grasslands Pitau	7 [ ]
very long	Aran	9 [ ]
<b>5.4 Leaf: width of median leaflet (12)</b>		
very narrow	Kent Wild White	1 [ ]
narrow	Barbian	3 [ ]
medium	Grasslands Huia	5 [ ]
broad	Grasslands Pitau	7 [ ]
very broad	Aran	9 [ ]



Characteristics		Example Varieties	Note
<b>5.5 Leaf: size of median leaflet [Proposed] (13)</b>			
very small		Kent Wild White	1 [ ]
small		Rivendel	3 [ ]
medium		Pertina	5 [ ]
large		Grasslands Pitau	7 [ ]
very large		Aran	9 [ ]
<b>6. Similar varieties and differences from these varieties</b>			
Denomination of similar variety	Characteristic in which the similar variety is different <sup>o)</sup>	State of expression of similar variety	State of expression of candidate variety
<p><sup>o)</sup> In the case of identical states of expressions of both varieties, please indicate the size of the difference.</p>			
<b>7. Additional information which may help to distinguish the variety</b>			
<b>7.1 Resistance to pest and diseases</b>			
<b>7.2 Special conditions for the examination of the variety</b>			
<b>7.3 Other information</b>			

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 that question is yes, please attach a copy of such an authorization.

[End of document]