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WORKING PAPER ON THE TEST GUIDELINES FOR RED CLOVER

*Document prepared by the experts from Germany*

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## I. Subject of these Guidelines

These Test Guidelines apply to all varieties of *Trifolium pratense* L.: lines and hybrid varieties.

## 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 there 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.

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. As a minimum, each test at each testing place should include per growing period:

(a) Row plots

3000 plants (density about 450 plants per m<sup>2</sup>) which should be divided between two replicates

(b) Plots with single spaced plants

60 single spaced plants which should be divided between three replicates.

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 observations for assessment of distinctness, uniformity and stability should be made:

- on 60 plants or part of plants, in case of single spaced plants

- on a minimum of 1500 plants in case of row plots.

The variability within the variety should not exceed the variability of comparable varieties already known.

#### 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:

Ploidy (characteristic 2)

#### 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 tests

M = actual measurement

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

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

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>1. C</b>	<b>Seed: color of coat</b>					
	yellow				Marino	1
	multicolored				Renova	2
	violet					3
<b>2. C</b> (* )	<b>Ploidy</b>					
	diploid				Renova	2
	tetraploid				Titus	4
<b>3. A</b> (+ ) <b>M</b>	<b>Cotyledon: length</b>					
	short				Wiro	3
	medium				Marino, Temara	5
	long				Maro, Maneta	7
<b>4. A</b> (+ ) <b>M</b>	<b>Cotyledon: width</b>					
	narrow				Wiro	3
	medium				Marino, Temara	5
	broad				Maro, Maneta	7
<b>5. B</b> (+ ) <b>VG</b>	<b>Plant: natural height in the year of sowing</b>					
	low					3
	medium				Maro	5
	high				Formica	7
<b>6. B</b> (+ ) <b>VG</b>	<b>Leaf: color in the year of sowing</b>					
	light green					3
	medium green				Rotra	5
	dark green				Tedi	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>7. A</b> <b>(+) M</b>	<b>Plant: tendency to flower in autumn of year of sowing</b>					
	erect					1
	semi-erecti					3
	intermediate				Rotra, Barfiola	5
	semi-prostrate					7
	prostrate				Lipiero, Wiro	9
<b>7a. A</b> <b>VG</b>	<b>Plant: tendency to flower in the year of sowing</b>					
	weak				Kora	3
	medium				Vivi, Sara	5
	strong				Barfiola	7
<b>8. B</b> <b>VG</b>	<b>Plant: natural height in spring</b>					
	low				Wiro	3
	medium				Silva	5
	high				Tedi	7
<b>9. B</b> <b>VG</b>	<b>Leaf: green color in spring</b>					
	light					3
	medium				Wiro	5
	dark				Lucrum	7
<b>10. A</b> <b>(*) M</b> <b>(+)</b>	<b>Time of flowering</b>					
	very early				Lipiero, Wiro	1
	early				Renova, Formica	3
	medium				Marino, Barfiola	5
	late				Lucrum, Markus	7
	very late				Kora, Björn	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>11. A</b> (*) <b>M</b> (+)	<b>Stem: length</b>					
		very short			Wiro	1
		short			Renova	3
		medium			Tempus	5
		long			Markus	7
	very long				9	
<b>12. A</b> (+) <b>M</b>	<b>Stem: thickness</b>					
		thin				3
		medium			Noe	5
	thick				7	
<b>13. A</b> (+) <b>M</b>	<b>Stem: number of internodes</b>					
		low				3
		medium				5
	high			Titus	7	
<b>14. A</b> (+) <b>M</b>	<b>Stem: density of hairs</b>					
		very low				1
		low			Lucrum	3
		medium				5
		high				7
	very high				9	
<b>15. A</b> (+) <b>M</b>	<b>Leaf: shape of medial leaflet</b>					
		longish				1
		oval			Tempus	2
	rounded				3	

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>16. A</b> <b>(*) M</b> <b>(+)</b>	<b>Leaf: length of medial leaflet</b>					
	short					3
	medium					5
	long					7
<b>17. A</b> <b>(*) M</b> <b>(+)</b>	<b>Leaf: width of medial leaflet</b>					
	narrow				Wiro	3
	medium				Merviot	5
	broad				Rotra	7
<b>18. A</b> <b>(*) M</b> <b>(+)</b>	<b>Leaf: intensity of white marks</b>					
	absent or very low					1
	low					3
	medium				Lucrum	5
	high				Temara	7
	very high					9
<b>19. B</b> <b>(+) VG</b>	Plant: natural height in aftermath					
	short				Lipiero	3
	medium				Markus	5
	high				Formica	7



VIII. Explanation on the Table of Characteristics

Ad. 2: Ploidy

Ploidy should be assessed on at least 100 seedlings.

Ad. 3: Cotyledon: length

Ad.4: Cotyledon: width

The observation should be made 12-14 days after sowing in greenhouse, when the first leaf is fully developed. If the two cotyledons differ in size, the biggest one should be measured.

Ad. 5: Plant: natural height in the year of sowing

Ad. 6: Leaf: color in the year of sowing

The observation should be made 4 to 5 weeks after the reduction cut.

Ad. 7: Plant: growth habit in autumn of year of sowing

A visual estimate is taken of the angle that the outer shoots make with the horizontal.

1 = erect

3 = semi-erect

5 = intermediate

7 = semi-prostrate

9 = prostrate

Ad. 10: Time of flowering

The observation is made when 3 heads per plant are flowering.

Ad. 11: Stem: length

Ad. 12: Stem: thickness

Ad. 13: Stem: number of internodes

The longest stem is observed including head within 1-2 weeks after mean date of flowering. The thickness is measured 3 to 5 cm above tillering node.

Ad. 14: Stem: density of hairs

The density of hairs should be observed on the 3<sup>rd</sup> internode of a fully expanded flower head on the same stem on which the length of stem is measured.

The observation should be made on this internode.

Ad. 15: Leaf: shape of medial leaflet

1  
longish

2  
ovate

3  
rounded

The observations should be made within 1-2 weeks after mean date of flowering on the 3<sup>rd</sup> leaf from top from the main stem.

Ad. 16: Leaf: length of medial leaflet

Ad. 17: Leaf: width of medial leaflet

Ad. 18: Leaf: intensity of white marks

The observations should be made within 1-2 weeks after mean date of flowering.

Ad. 19: Plant: natural height in aftermath

The observation should be made within 4 to 6 weeks after the summer cut.

IX. Literature

Taylor, N.L., 1985 "Clover science et technology", Agronomy nr. 25 in the series American Society of Agronomy, Inc., Crop Science Society

Taylor, N.L. and Quesenberry, K.H., 1996. Red Clover Science, Kluwer Academic Publishers, 228pp.

Mousset-Déclas, C., 1992. Le Trèfle Violet. In "Amélioration des espèces végétales cultivées, objectif et critères de sélection, ed. Gallais et Bannerot, INRA ed., pp.339-348

Mousset-Déclas, C., 1995. Les trèfles ou le genre Trifolium. In "ressources Génétiques des Plantes Fourragères et à gazon. Properi, Guy, Balfourier Coord. Coéd. BRG-INRA, pp. 177-211.

X. Technical Questionnaire

	Reference Number (not to be filled in by the applicant)
<p><b>TECHNICAL QUESTIONNAIRE</b> to be completed in connection with an application for plant breeders' rights</p>	
1. Species	<p style="text-align: center;"><i>Trifolium pratense</i> L.  RED CLOVER</p>
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 Ploidy</b> (2)		
diploid	Renova	2 [ ]
tetraploid	Titus	4 [ ]
<b>5.2 Time of flowering</b> (10)		
very early	Lipiero, Wiro	1 [ ]
early	Renova, Formica	3 [ ]
medium	Marino, Barfiola	5 [ ]
late	Lucrum, Markus	7 [ ]
very late	Kora, Björn	9 [ ]
<b>5.3 Stem: length</b> (11)		
very short	Wiro	1 [ ]
short	Renova	3 [ ]
medium	Tempus	5 [ ]
long	Markus	7 [ ]
very long		9 [ ]
<b>5.4 Leaf: length of medial leaflet</b> (16)		
short	Wiro	3 [ ]
medium	Renova	5 [ ]
long	Tedi	7 [ ]

Characteristics		Example Varieties	Note
<b>5.5 Leaf: width of medial leaflet (17)</b>			
narrow		Wiro	3 [ ]
medium		Merviot	5 [ ]
broad		Rotra	7 [ ]
6. Similar varieties and differences from these varieties			
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
<sup>o)</sup> In the case of identical states of expressions of both varieties, please indicate the size of the difference.			



