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INTERNATIONAL UNION FOR THE PROTECTION OF NEW VARIETIES OF PLANTS

Geneva

DRAFT

RED CLOVER

UPOV Code(s): TRFOL_PRA

Trifolium pratense L.

GUIDELINES

FOR THE CONDUCT OF TESTS

FOR DISTINCTNESS, UNIFORMITY AND STABILITY

prepared by experts from South Africa

to be considered by the

Technical Working Party for Agricultural Crops
at its forty-fifth session, to be held in Mexico City, Mexico,
from 2016-07-11 to 2016-07-15

Disclaimer: this document does not represent UPOV policies or guidance

Alternative names:*

Botanical name	*English*	*French*	*German*	*Spanish*
Trifolium pratense L.	Red Clover	Trèfle violet	Rotklee	Trebol rojo, Trébol violeta

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.]

TABLE OF CONTENTS PAGE

1. Subject of these Test Guidelines

These Test Guidelines apply to all varieties of *Trifolium pratense* 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 seeds.

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.

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

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

3.1.2 The two independent growing cycles should be in the form of two separate plantings.

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

3.3.1 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.

3.3.2 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 plots
C: special tests

3.3.4 a) In cases with extreme winter conditions sowing can be done in spring, with a reduction cut in early autumn.

b) In cases with a milder winter climate, sowing can be done in autumn and evaluations completed by early summer with no reduction cut of the plants.

3.4 Test Design

3.4.1 Row plots: Each test should be designed to result in a total of at least 3000 plants , which should be divided between at least 2 replicates.

3.4.2 Plots with single spaced plants: Each test should be design to result in a total of at least 60 plants which should be divided between at least 3 replicates.

3.4.3 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 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.

4.1.4 Number of plants or parts of plants to be Examined

Unless otherwise indicated, for the purposes of distinctness, all observations on single plants should be made on 60 plants or parts of plants taken from each of 60 plants and any other observations made on all plants in the test, disregarding any off-type plants.

In the case of observations of parts taken from single plants, the number of parts to be taken from each of the plants should be 1.

4.1.5 Method of Observation

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

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

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

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

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

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

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

4.2 Uniformity

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 further examined by testing a new seed stock to ensure that it exhibits the same characteristics as those shown by the initial material supplied.

5. Grouping of Varieties and Organization of the Growing Trial

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

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

5.3 The following have been agreed as useful grouping characteristics:

(a) Plant: ploidy (characteristic 2)
(b) Time of flowering (characteristic 24)

5.4 Guidance for the use of grouping characteristics, in the process of examining distinctness, is provided through the General Introduction and document TGP/9 “Examining Distinctness”.

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

6.2.1 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.

6.2.2 In the case of qualitative and pseudo-qualitative characteristics (see Chapter 6.3), all relevant states of expression are presented in the characteristic. However, in the case of quantitative characteristics with 5 or more states, an abbreviated scale may be used to minimize the size of the Table of Characteristics. For example, in the case of a quantitative characteristic with 9 states, the presentation of states of expression in the Test Guidelines may be abbreviated as follows:

State	*Note*
small	3
medium	5
large	7

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

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

6.2.3 Further explanation of the presentation of states of expression and notes is provided in document TGP/7 “Development of Test Guidelines”.

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

	English	français	deutsch	español	Example Varieties Exemples Beispielssorten Variedades ejemplo	Note/ Nota
1 2	**3 4**	**5 6**	**7**			
	Name of characteristics in English	**Nom du caractère en français**	**Name des Merkmals auf Deutsch**	**Nombre del carácter en español**		
	states of expression	types d'expression	Ausprägungsstufen	tipos de expresión		

1 Characteristic number

2 (*) Asterisked characteristic – see Chapter 6.1.2

3 Type of expression
QL Qualitative characteristic – see Chapter 6.3
QN Quantitative characteristic – see Chapter 6.3
PQ Pseudo-qualitative characteristic – see Chapter 6.3

4 Method of observation (and type of plot, if applicable)
MG, MS, VG, VS – see Chapter 4.1.5

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

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

7 Not applicable

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

		English	français	deutsch	español	Example Varieties Exemples Beispielssorten Variedades ejemplo	Note/ Nota
1.		**PQ VG**					
		Seed : color of coat					
		yellow				Marino	1
		yellow to orange				Atlantis, Harmonie	2
		multicolored				Renova	3
2.	**(*)**	**QL VS**	**(+)**				
		Plant: ploidy					
		diploid				Renova	2
		tetraploid				Titus	4
3.		**QN MS|C**	**(a)**				
		Cotyledon: length					
		short				Wiro	3
		medium				Marino, Temara	5
		long				Maneta, Maro	7
4.		**QN MS|C**	**(a)**				
		Cotyledon: width					
		narrow				Wiro	3
		medium				Marino, Temara	5
		broad				Maneta, Maro	7
5.		**QN MG|B**	**(+)**				
		Plant: natural height <u>without</u> vernalization					
		short					3
		medium				Marino	5
		tall				Formica	7

		English	français	deutsch	español	Example Varieties Exemples Beispielssorten Variedades ejemplo	Note/ Nota
6.	**(*)**	**QN VS|A**	**(+) (b)**				
		Plant: growth habit <u>without</u> vernalization					
		erect				Red Gold	1
		semi-erect				Regal	3
		intermediate				Barfiola, Rotra	5
		semi-prostrate				Board	7
		prostrate				Banduro, Lipiero, Rubitas, Wiro	9
7.	**(*)**	**QN VG|B**	**(+) (b)**				
		Plant: tendency to flower <u>without</u> vernalization					
		weak				Kora	3
		medium				Sara, Vivi	5
		strong				Barfiola	7
8.	**(*)**	**QN VG|B**	**(+) (b)**				
		Plant: natural height <u>after</u> vernallization					
		short				Wiro	3
		medium				Silva	5
		tall				Tedi	7
9.		**QN VS|B**					
		Plant: density of foilage in vegetative phase					
		very low					1
		low					3
		medium					5
		high					7
		very high					9

		English	français	deutsch	español	Example Varieties Exemples Beispielssorten Variedades ejemplo	Note/ Nota
10.		**QN MS|A**	**(+) (b)**				
		Stem: length					
		very short				Wiro	1
		short				Renova	3
		medium				Tempus	5
		long				Markus	7
		very long					9
11.		**QN MS|A**	**(+) (b)**				
		Stem: thickness					
		thin				Banduro	3
		medium				Noe	5
		thick					7
12.	**(*)**	**QN MS|A**		**(b)**			
		Stem: number of internodes					
		low					3
		medium					5
		high				Titus	7
13.		**QN VS|A**	**(+) (b)**				
		Stem: density of hairs					
		very low					1
		low				Lucrum	3
		medium					5
		high					7
		very high					9
14.		**QN VG|A**		**(b)**			
		Stem: intensity of anthocyanin colour					
		weak				Rubitas	3
		medium				Board	5
		strong				Renegade	7

		English	français	deutsch	español	Example Varieties Exemples Beispielssorten Variedades ejemplo	Note/ Nota
15.		**QN VG|B**	**(+)**				
		Leaf: intensity of green color <u>without</u> vernalization					
		light				Kenland	3
		medium				Rotra	5
		dark				Tedi	7
16.	**(*)**	**QN VG|B**	**(+)**	**(b)**			
		Leaf: intensity of green color <u>after</u> vernalization					
		light				Renegade	3
		medium				Freedom, Wiro	5
		dark				Lucrum, Rubitas	7
17.		**QN MS|B**					
		Leaf: length of petiole					
		short					3
		medium					5
		long					7
18.		**QN MS|B**					
		Leaf: thickness of petiole					
		very thin					1
		thin					2
		medium					3
		thick					4
		very thick					5
19.	**(*)**	**QN VG|A**	**(+)**	**(b)**			
		Leaf: relative area of leaf markings					
		absent or very small				Lemmon	1
		small				Kenland	2
		medium				Banduro	3
		large					4
		very large				Rubitas	5

		English	français	deutsch	español	Example Varieties Exemples Beispielssorten Variedades ejemplo	Note/ Nota
20.	**(*)**	**QN VG|A**	**(+)** **(b)**				
		Leaf: intensity of leaf markings					
		weak				Board	1
		medium				Lucrum	2
		strong				Rubitas, Temara	3
21.	**(*)**	**PQ VG|A**	**(+)** **(b)**				
		Median leaflet: shape					
		ovate				Tempus	1
		elliptic				Lemmon	2
		circular					3
22.	**(*)**	**QN MS|A**	**(b)**				
		Median leaflet: length					
		short					3
		medium					5
		long					7
23.	**(*)**	**QN MS|A**	**(b)**				
		Median leaflet: width					
		narrow				Wiro	3
		medium				Merviot	5
		broad				Rotra	7
24.	**(*)**	**QN MS|A**	**(+)**				
		Time of flowering					
		very early				Lipiero, Wiro	1
		early				Formica, Renova	3
		medium				Barfiola, Marino	5
		late				Lucrum, Markus	7
		very late				Bjorn, Kora	9

	English	français	deutsch	español	Example Varieties Exemples Beispielssorten Variedades ejemplo	Note/ Nota
25.	**QN** **MS|B**					
	Inflorescence: length of peduncle					
	short					3
	medium					5
	long					7
26.	**QN** **MS|B**					
	Inflorescence: thickness of peduncle					
	very thin					1
	thin					2
	medium					3
	thick					4
	very thick					5

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) Observations on the length and width of the Cotyledon should be made 12-14 days after sowing in the greenhouse, when the first leaf is fully developed. If the two cotyledons differ in size, the biggest one should be measured.

(b) All observations to be done approximately 1-2 weeks after mean flowering date unless otherwise indicated.

8.2 Explanations for individual characteristics

Ad. 2: Plant: ploidy

Ploidy should be determined by standard cytological methods.

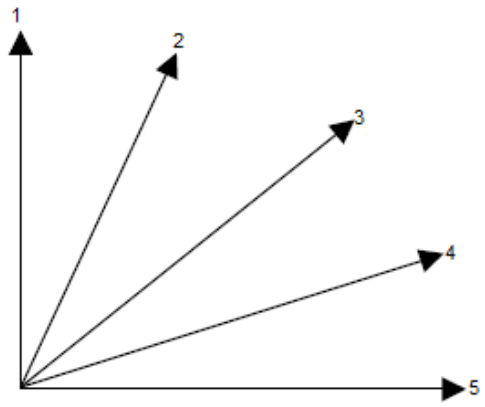
Ad. 5: Plant: natural height without vernalization

The observation should be made before the reduction cut

Ad. 6: Plant: growth habit without vernalization

Observations on growth habit to be made before the reduction cut

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



1 = erect

2 = semi-erect

3 = intermediate

4 = semi-prostrate

5 = prostrate

Ad. 7: Plant: tendency to flower without vernalization

The observation should be made before the reduction cut.

Ad. 8: Plant: natural height after vernallization

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

Ad. 10: Stem: length

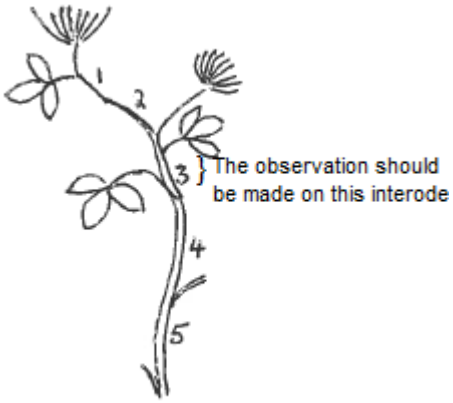
The longest stem should be observed including the flower head.

Ad. 11: Stem: thickness

The thickness should be measured 2 to 4 cm above the tillering node.

Ad. 13: Stem: density of hairs

Should be observed on the 3rd internode as indicated in the pitcure below.



Ad. 15: Leaf: intensity of green color without vernalization

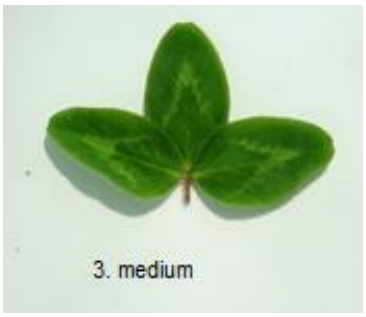
The observation should be made before the reduction cut.

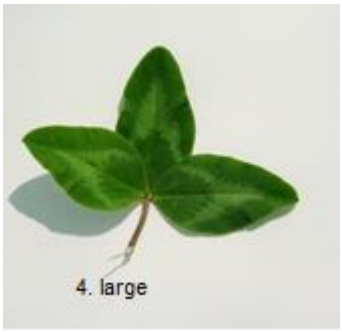
Ad. 16: Leaf: intensity of green color after vernalization

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

Ad. 19: Leaf: relative area of leaf markings

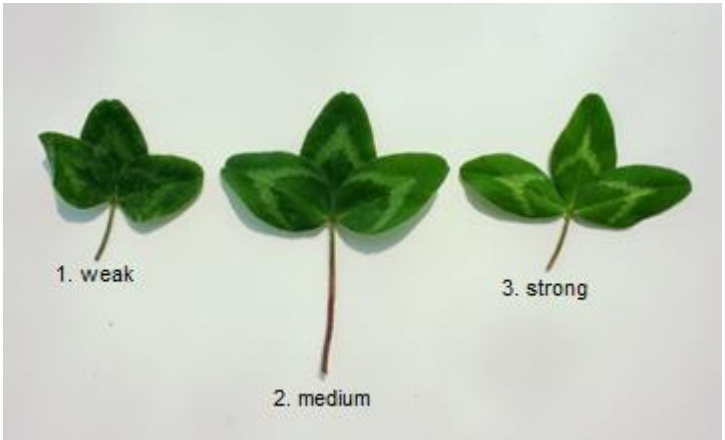
Observations should be made at the beginning of flowering



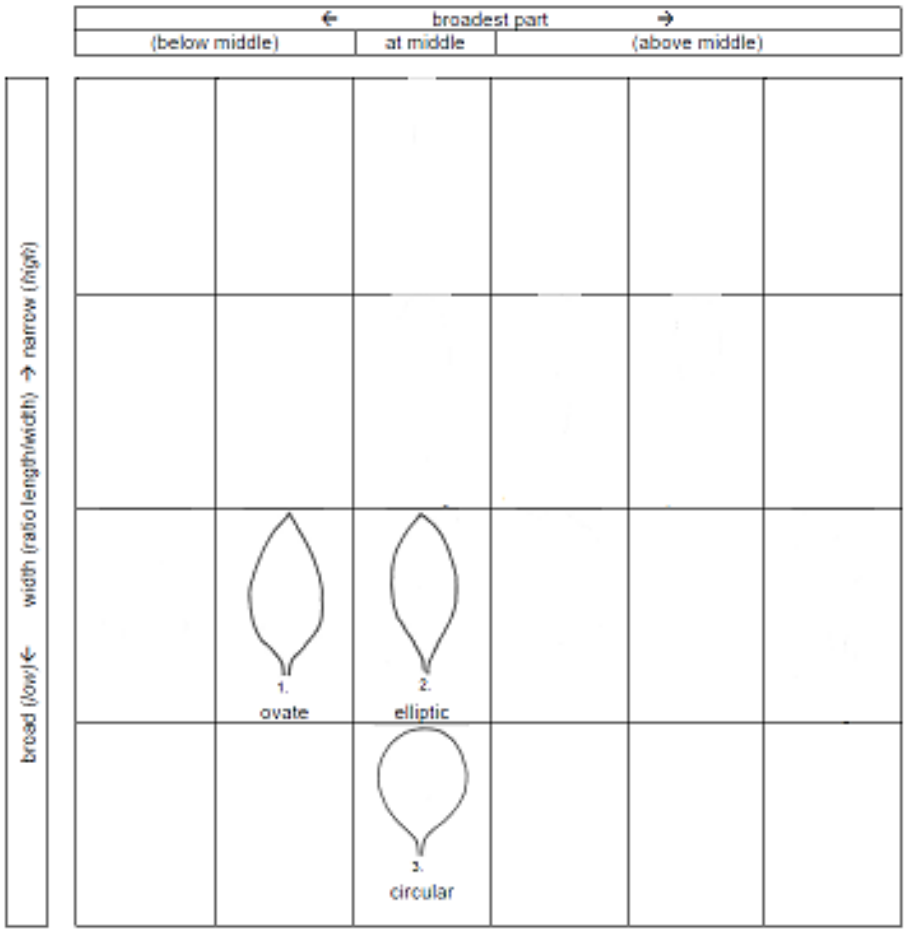


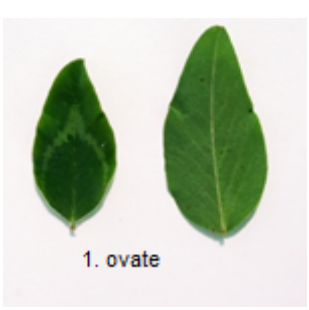
Ad. 20: Leaf: intensity of leaf markings

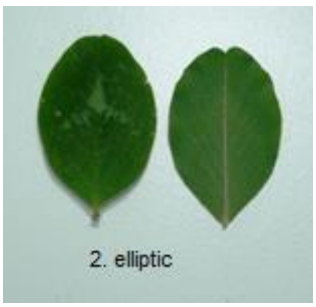
The observation should be made at beginning of flowering



Ad. 21: Median leaflet: shape







Ad. 24: Time of flowering

The observation should be made when 3 flowers per plant are open.

9. Literature

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Taylor, N.L., 1985: “Clover science and 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, 228 pp.

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. Subject of the Technical Questionnaire

1.1	Botanical name	*Trifolium pratense* L.
1.2	Common name	Red 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 {x} of {y}	Reference Number:

#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)

(……..……..…) x (……..……..…)

female parent male parent

(b) partially known cross [ ]

(please state known parent variety(ies))

(……..……..…) x (……..……..…)

female parent male parent

(c) unknown cross [ ]

4.1.2 Mutation [ ]

(please state parent variety)

4.1.3 Discovery and development [ ]

(please state where and when discovered and how developed)

4.1.4 Other [ ]

(please provide details)

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

4.2 Method of propagating the variety

4.2.1 Other [ ]
(Please provide details)

TECHNICAL QUESTIONNAIRE	Page {x} of {y}	Reference Number:

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	**Plant: ploidy**		
(2)			
	diploid	Renova	2 [ ]
	tetraploid	Titus	4 [ ]
5.2	**Stem: length**		
(10)			
	very short	Wiro	1 [ ]
	short	Renova	3 [ ]
	medium	Tempus	5 [ ]
	long	Markus	7 [ ]
	very long		9 [ ]
5.3	**Median leaflet: length**		
(22)			
	short		3 [ ]
	medium		5 [ ]
	long		7 [ ]
5.4	**Median leaflet: width**		
(23)			
	narrow	Wiro	3 [ ]
	medium	Merviot	5 [ ]
	broad	Rotra	7 [ ]
5.5	**Time of flowering**		
(24)			
	very early	Lipiero, Wiro	1 [ ]
	early	Formica, Renova	3 [ ]
	medium	Barfiola, Marino	5 [ ]
	late	Lucrum, Markus	7 [ ]
	very late	Bjorn, Kora	9 [ ]

TECHNICAL QUESTIONNAIRE	Page {x} of {y}	Reference Number:

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
Example			

Comments:

TECHNICAL QUESTIONNAIRE	Page {x} of {y}	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?

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

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

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.

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 for where you have indicated “yes”.

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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]