



TG/170/3

INTERNATIONAL UNION  
FOR THE PROTECTION  
OF NEW VARIETIES OF  
PLANTS

UNION INTERNATIONALE  
POUR LA PROTECTION  
DES OBTENTIONS  
VÉGÉTALES

INTERNATIONALER  
VERBAND ZUM SCHUTZ  
VON PFLANZEN-  
ZÜCHTUNGEN

UNIÓN INTERNACIONAL  
PARA LA PROTECCIÓN  
DE LAS OBTENCIONES  
VEGETALES

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

**SUBTERRANEAN  
CLOVER**  
*(Trifolium subterraneum)*

**GENEVA**  
**2001**

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## **GUIDELINES**

### **FOR THE CONDUCT OF TESTS**

#### **FOR DISTINCTNESS, UNIFORMITY AND STABILITY**

#### **SUBTERRANEAN CLOVER**

*(Trifolium subterraneum)*

These Guidelines should be read in conjunction with document TG/1/2, which contains explanatory notes on the general principles on which the Guidelines have been established.

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## I. Subject of these Guidelines

These Test Guidelines apply to all varieties of *Trifolium subterraneum* (ssp. *subterraneum*, ssp. *yanninicum* and ssp. *brachycalycinum*).

## 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:

100 g.

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 seed should be inoculated with the appropriate strain of Rhizobium for *Trifolium subterraneum*. 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 should include 30 spaced plants and may in addition include 4 meters of row. Separate plots for observation and for measuring can only be used if they have been subject to similar environmental conditions.
4. Plots with spaced plants: Each test should consist of 30 single spaced plants per variety arranged in 2, 3, or 5 replicates, i.e. plots of 15, 10 or 6 plants.
5. Row plots: Each test which includes row plots should consist of at least 4 meters of row arranged in two replicates, each of 2 meters. The size of the plots should be such that plants or parts of plants may be removed for observation without prejudice to the visual assessments which must be made up to the end of the growing period. The density of sowing should be such that about 150 plants per meter should be obtained.
6. Additional tests for special purposes may be established.

#### IV. Methods and Observations

1. All measurements for assessment of distinctness and stability should be made on 30 plants or parts taken from each of 30 plants.
2. For the assessment of uniformity, a population standard of 1% with an acceptance probability of at least 95% should be applied. In the case of a sample size of 30 plants, the maximum number of off-types allowed would be 1.
3. Unless indicated otherwise, all observations on the leaf should be made on new fully opened leaves at the 50% flowering stage (50% of plants with at least one flower). Observations on flowers should be made 2 weeks after the 50% flowering stage. Observations on the burr and seed should be made on fully mature, senesced plants.

#### 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. In the first place, the collection should be divided according to the subspecies:
  - *subterraneum*
  - *yannicum* or
  - *brachycalycinum*.
3. It is recommended that the competent authorities use the following characteristics for grouping varieties within each subspecies:
  - (a) Leaflet: pattern of mark (characteristic 6)
  - (b) Stipules: degree of anthocyanin coloration (in shaded part of canopy) (characteristic 29)
  - (c) Time of start of flowering (characteristic 30)
  - (d) Calyx tube: distribution of coloration (characteristic 34)
  - (e) Stem (runner): degree of hairiness (internode between the third and fourth node on the longest primary branch) (characteristic 36)
  - (f) Seed: hard seed breakdown over four months (characteristic 43).

#### 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

MS = actual measurement

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> Parzelle <sup>1)</sup> Parcela <sup>1)</sup>	English	français	deutsch	español	Example Varieties Exemples Beispielssorten Variedades ejemplo	Note/ Nota
<b>1. A VS</b>	<b>Leaf: hairiness of petiole</b>	<b>Feuille: pilosité du pétiole</b>	<b>Blatt: Behaarung des Stieles</b>	<b>Hoja: velloidad del peciolo</b>		
	absent or very weak	absente ou très faible	fehlend oder sehr gering	ausente o muy débil	Denmark, Larisa	1
	weak	faible	gering	débil	Clare	3
	medium	moyenne	mittel	media	Dalkeith, Esperance	5
	strong	forte	stark	fuerte	Bacchus Marsh	7
	very strong	très forte	sehr stark	muy fuerte		9
<b>2. A VS</b>	<b>Leaf: attitude of petiole hairs</b>	<b>Feuille: port des poils du pétiole</b>	<b>Blatt: Stellung der Haare des Stieles</b>	<b>Hoja: porte de los pelos del peciolo</b>		
	erect	dressé	aufrecht	erecto	Mt Barker	1
	semi-erect	demi-dressé	halbaufrecht	semi-erecto	Dalkeith	2
	adpressed	appliqué	anliegend	tumbado		3
<b>3. A MS</b>	<b>Leaflet: ratio maximum length/maximum width</b>	<b>Foliolo: rapport longueur maximale/largeur maximale</b>	<b>Fiederblatt: Verhältnis maximale Länge/maximale Breite</b>	<b>Folíolo: razón longitud máxima/anchura máxima</b>		
	very small	très petit	sehr klein	muy pequeño	Meteora, Mt. Baker, Northam	1
	small	petit	klein	pequeño	Seaton Park, York	3
	medium	moyen	mittel	medio	Geraldton	5
	large	grand	groß	grande		7
	very large	très grand	sehr groß	muy grande		9
<b>4. A VS (+)</b>	<b>Leaflet: general shape</b>	<b>Foliolo: forme générale</b>	<b>Fiederblatt: allgemeine Form</b>	<b>Folíolo: forma general</b>		
	triangular	triangulaire	dreieckig	triangular	Geraldton, Yarloop	1
	triangular to rounded	triangulaire à ronde	dreieckig bis rund	triangular a redondeada	Seaton Park	2
	rounded	ronde	rund	redondeada	Meteora, Northam	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>5. B VG</b>	<b>Leaflet: intensity of green color</b>	<b>Foliolle: intensité de la couleur verte</b>	<b>Fiederblatt: Intensität der Grünfärbung</b>	<b>Folíolo: intensidad del color verde</b>		
	light	claire	hell	claro		3
	medium	moyenne	mittel	medio		5
	dark	foncée	dunkel	oscuro	Dalkeith, Leura	7
<b>6. A (* VS</b>	<b>Leaflet: pattern of mark</b>	<b>Foliolle: disposition du dessin</b>	<b>Fiederblatt: Verteilung der Markierung</b>	<b>Folíolo: patrón de la marca</b>		
	a pair of arms only	seulement une paire de bras	nur ein Paar Streifen	sólo un par de brazos	Yarloop	1
	a single transverse band only	seulement une bande transversale	nur ein einzelnes transversales Band	sólo una banda transversal	Nungarin	2
	a single, crescent-shaped central mark only	un seul dessin central en forme de croissant	nur eine einzelne halbmondförmige Markierung	una sola marca central en forma de luna creciente	Mt Barker	3
	a pair of arms and a crescent	une paire de bras et un croissant	ein Paar Streifen und ein Halbmond	un par de brazos y una luna creciente	Seaton Park	4
<b>7. A (+ VS</b>	<b><u>Only for varieties with arms:</u> Leaflet: width of arms</b>	<b><u>Seulement pour variétés avec bras:</u> Foliolle: largeur des bras</b>	<b><u>Nur für Sorten mit Streifen:</u> Fiederblatt: Breite der Streifen</b>	<b><u>Sólo para variedades con brazos:</u> Folíolo: anchura de los brazos</b>		
	narrow	étroits	schmal	estrecha	Dalkeith, Leura, Yarloop	3
	medium	moyens	mittel	media	Junea, Seaton Park	5
	broad	larges	breit	ancha		7
<b>8. A VS</b>	<b><u>Only for varieties with arms:</u> Leaflet: clarity of arms</b>	<b><u>Seulement pour variétés avec bras:</u> Foliolle: netteté des bras</b>	<b><u>Nur für Sorten mit Streifen:</u> Fiederblatt: Deutlichkeit der Streifen</b>	<b><u>Sólo para variedades con brazos:</u> Folíolo: claridad de los brazos</b>		
	faint	faible	schwach	débil	Denmark, Yarloop	1
	clear	claire	deutlich	clara	Seaton Park	2

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>9. A VS</b>	<b><u>Only for varieties with arms:</u> Leaflet: color of arms</b>	<b><u>Seulement pour variétés avec bras:</u> Foliole: couleur des bras</b>	<b><u>Nur für Sorten mit Streifen:</u> Fiederblatt: Farbe der Streifen</b>	<b><u>Sólo para variedades con brazos:</u> Folículo: color de los brazos</b>		
	white	blancs	weiß	blanco	Junea, Leura, Seaton Park	1
	cream	crème	cremefarben	crema	Karridale	2
	light green	vert clair	hellgrün	verde claro	Denmark, Rosedale, Woogenellup	3
	medium green	vert moyen	mittelgrün	verde medio	Nuba	4
	red	rouges	rot	rojo		5
	purple	pourpres	purpur	púrpura		6
	brown	bruns	braun	marrón		7
<b>10. A VS (+)</b>	<b><u>Only for varieties with bands:</u> Leaflet: width of bands</b>	<b><u>Seulement pour variétés avec bandes:</u> Foliole: largeur des bandes</b>	<b><u>Nur für Sorten mit Bändern:</u> Fiederblatt: Breite der Bänder</b>	<b><u>Sólo para variedades con bandas:</u> Folículo: grosor de las bandas</b>		
	narrow	étroites	schmal	estrecha	Geraldton, Northam	3
	medium	moyennes	mittel	media	Nungarin	5
	broad	larges	breit	ancha		7
<b>11. A VS</b>	<b><u>Only for varieties with bands:</u> Leaflet: clarity of bands</b>	<b><u>Seulement pour variétés avec bandes:</u> Foliole: clarté des bandes</b>	<b><u>Nur für Sorten mit Bändern:</u> Fiederblatt: Deutlichkeit der Bänder</b>	<b><u>Sólo para variedades con bandas:</u> Folículo: claridad de las bandas</b>		
	faint	faible	undeutlich	débil		1
	clear	claire	deutlich	clara	Geraldton, Nungarin	2

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
12. A VS	<b><u>Only for varieties with bands:</u></b> Leaflet: color of bands	<b><u>Seulement pour variétés avec bandes:</u></b> Foliole: couleur des bandes	<b><u>Nur für Sorten mit Bändern:</u></b> Fiederblatt: Farbe der Bänder	<b><u>Sólo para variedades con bandas:</u></b> Folíolo: color de las bandas		
	white	blanches	weiß	blanca		1
	cream	crème	cremefarben	crema		2
	light green	vert clair	hellgrün	verde clara	Geraldton, Northam, Nungarin	3
	medium green	vert moyen	mittelgrün	verde medio		4
	red	rouges	rot	roja		5
	purple	pourpres	purpur	púrpura		6
	brown	brunes	braun	marrón		7
13. A VS	<b><u>Only for varieties with bands:</u></b> Leaflet: position of bands	<b><u>Seulement pour variétés avec bandes:</u></b> Foliole: position des bandes	<b><u>Nur für Sorten mit Bändern:</u></b> Fiederblatt: Stellung der Bänder	<b><u>Sólo para variedades con bandas:</u></b> Folíolo: posición de las bandas		
	towards base	vers la base	zur Basis hin	hacia la base		1
	central	centrale	zentral	central	Geraldton, Northam, Nungarin	2
towards distal end	vers le sommet	zur Spitze hin	hacia el extremo distal		3	
14. A VS	<b><u>Only for varieties with crescent:</u></b> Leaflet: position of crescent	<b><u>Seulement pour variétés avec croissant:</u></b> Foliole: position du croissant	<b><u>Nur für Sorten mit Halbmond:</u></b> Fiederblatt: Stellung des Halbmonds	<b><u>Sólo para variedades con bandas:</u></b> Folíolo: posición de la medialuna		
	towards base	vers la base	zur Basis hin	hacia la base		1
	central	centrale	zentral	central	Mt Barker, Seaton Park	2
towards distal end	vers le sommet	zur Spitze hin	hacia el extremo distal	Meteora, Riverina	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
15. A VS	<b><u>Only for varieties with both a crescent and arms:</u></b> <b>Leaflet: position of arms relative to crescent</b>	<b><u>Seulement pour variétés avec un croissant et des bras:</u></b> <b>Foliolo: position des bras par rapport au croissant</b>	<b><u>Nur für Sorten mit sowohl einem Halbmond als auch einem Streifen:</u></b> <b>Fiederblatt: Stellung des Streifens im Vergleich zum Halbmond</b>	<b><u>Sólo para variedades con medialuna y brazos:</u></b> <b>Folíolo: posición de los brazos relativa a la medialuna</b>		
	arms adjacent only to crescent	bras contigus seulement au croissant	Streifen nur am Halbmond angrenzend	brazos contiguos solamente a la medialuna	Junee, Leura, Trikkala	1
	arms both adjacent and beneath crescent	bras contigus et en dessous du croissant	Streifen sowohl am Halbmond angrenzend als auch unter dem Halbmond	brazos al mismo tiempo contiguos y debajo la medialuna	Karridale, Seaton Park	2
16. A VS (+)	<b><u>Only for varieties with crescent:</u></b> <b>Leaflet: base of crescent</b>	<b><u>Seulement pour variétés avec croissant:</u></b> <b>Foliolo: base du croissant</b>	<b><u>Nur für Sorten mit Halbmond:</u></b> <b>Fiederblatt: Basis des Halbmonds</b>	<b><u>Sólo para variedades con medialuna:</u></b> <b>Folíolo: base de la medialuna</b>		
	Type C1	Type C1	Typ C1	Tipo C1	Daliak	1
	Type C2	Type C2	Typ C2	Tipo C2	Dalkeith, Junee	2
	Type C3	Type C3	Typ C3	Tipo C3	Mt Barker	3
	Type C4	Type C4	Typ C4	Tipo C4	Meteora	4
17. A VS	<b><u>Only for varieties with crescent:</u></b> <b>Leaflet: color of crescent</b>	<b><u>Seulement pour variétés avec croissant:</u></b> <b>Foliolo: couleur du croissant</b>	<b><u>Nur für Sorten mit Halbmond:</u></b> <b>Fiederblatt: Farbe des Halbmonds</b>	<b><u>Sólo para variedades con medialuna:</u></b> <b>Folíolo: color de la medialuna</b>		
	white	blanc	weiß	blanco		1
	cream	crème	cremefarben	crema		2
	light green	vert clair	hellgrün	verde claro		3
	medium green	vert moyen	mittelgrün	verde medio	Mt. Baker, Riverina, Seaton Park	4
	red	rouge	rot	rojo		5
	purple	pourpre	purpur	púrpura		6
	brown	brun	braun	marrón		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>18. A VS</b>	<b>Leaflet: indentation of distal margin</b>	<b>Foliolo: denticulation du bord distal</b>	<b>Fiederblatt: Zähnung des distalen Randes</b>	<b>Folíolo: dentado del borde distal</b>		
	absent or very weak	absente ou très faible	fehlend oder sehr gering	ausente o muy débil	Dwalganup	1
	weak	faible	gering	débil		3
	medium	moyenne	mittel	medio	Dalkeith, Seaton Park	5
	strong	forte	stark	fuerte	Woogenellup	7
	very strong	très forte	sehr stark	muy fuerte		9
<b>19. A VS</b>	<b>Leaflet: degree of anthocyanin flecks</b>	<b>Foliolo: importance des taches anthocyaniques</b>	<b>Blattfieder: Ausprägung der Anthocyanflecke</b>	<b>Folíolo: grado de las manchas antociánicas</b>		
	absent or very weak	nulle ou très faible	fehlend oder sehr gering	ausente o muy débil	Seaton Park	1
	weak	faible	gering	débil	Dalkeith, Woogenellup	3
	medium	moyenne	mittel	media	Daliak	5
	strong	forte	stark	fuerte	Mt. Barker	7
	very strong	très forte	sehr stark	muy fuerte	Bacchus Marsh	9
<b>20. A VS</b>	<b>Leaf: position of anthocyanin flecks</b>	<b>Feuille: position des taches anthocyaniques</b>	<b>Blatt: Position der Anthocyanflecke</b>	<b>Folíolo: posición de las manchas antociánicas</b>		
	predominately on upper surface	le plus souvent sur la face supérieure	überwiegend auf der Oberseite	predominantemente en la cara superior		1
	predominately on lower surface	le plus souvent sur la face inférieure	überwiegend auf der Unterseite	predominantemente en la cara inferior		2
	on both sides	sur les deux côtés	auf beiden Seiten	en ambas caras		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>21. A VS</b>	<b>Leaflet: degree of flush</b>	<b>Foliole: degré des traces diffuses</b>	<b>Fiederblatt: Ausprägung der Flammung</b>	<b>Folíolo: grado de la traza</b>		
	absent or very weak	nul ou très faible	fehlend oder sehr gering	ausente o muy débil	Dalkeith, Denmark	1
	weak	faible	gering	débil	Leura, Nungarin	3
	medium	moyen	mittel	media	Dinninup, Dwalganup	5
	strong	fort	stark	fuerte	Clare	7
	very strong	très fort	sehr stark	muy fuerte		9
<b>22. A VS</b>	<b>Leaflet: color of flush</b>	<b>Foliole: couleur des traces diffuses</b>	<b>Fiederblatt: Farbe der Flammung</b>	<b>Folíolo: color de la traza</b>		
	red	rouge	rot	rojo		1
	pinkish-brown	brun rosâtre	rosabraun	marrón rosa		2
	brown	brun	braun	marrón	Dinninup, Yarloop	3
	purplish-brown	brun pourpre	purpurbraun	marrón púrpura	Dwalgnup, Trikkala	4
	reddish-purple	pourpre-rougeâtre	rötlich purpur	púrpura rojizo	Gosse, Riverina	5
	brownish-purple	pourpre-brunâtre	bräunlich purpur	púrpura parduzco	Clare	6
	purple	pourpre	purpur	púrpura		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>23. A VS</b>	<b>Leaflet: predominant location of flush</b>	<b>Foliolle: position prédominante des traces diffuses</b>	<b>Fiederblatt: überwiegende Position der Flammung</b>	<b>Folíolo: posición predominante de la traza</b>		
	between distal end and leaf mark	entre l'extrémité distale et le dessin de la feuille	zwischen distalem Ende und der Blattmarkierung	entre el extremo distal y la marca de la hoja	Meteora	1
	around leaf mark only	seulement autour du dessin de la feuille	nur um die Blattmarkierung herum	sólo alrededor de la marca de la hoja	Enfield	2
	along midrib only	seulement le long de la nervure principale	nur entlang der Mittelrippe	sólo a lo largo del nervio principal	Yarloop	3
	along midrib and around leaf mark	le long de la nervure principale et autour du dessin de la feuille	entlang der Mittelrippe und um die Blattmarkierung herum	a lo largo del nervio principal y alrededor de la marca de la hoja	Dinninup	4
between leaf mark and base	entre le dessin de la feuille et la base	zwischen der Blattmarkierung und der Basis	entre la marca de la hoja y la base	Clare, Trikkala	5	
<b>24. A VS</b>	<b>Leaflet: degree of hairiness of upper surface</b>	<b>Foliolle: degré de la pilosité de la face supérieure</b>	<b>Fiederblatt: Ausprägung der Behaarung der Oberseite</b>	<b>Folíolo: grado de la vellosidad de la cara superior</b>		
	absent or very weak	nul ou très faible	fehlend oder sehr gering	ausente o muy débil	Clare, Dinninup, Gosse	
	weak	faible	gering	débil	Denmark, Goulburn	3
	medium	moyen	mittel	media	Dalkeith, Rosedale	5
	strong	fort	stark	fuerte	Leura, Nungarin	7
very strong	très fort	sehr stark	muy fuerte		9	
<b>25. A VS</b>	<b>Leaflet: attitude of hairs of upper surface</b>	<b>Foliolle: port des poils de la face supérieure</b>	<b>Fiederblatt: Stellung der Haare der Oberseite</b>	<b>Folíolo: porte de los pelos de la cara superior</b>		
	erect	dressé	aufrecht	erectos	Mt Barker	1
	semi-erect	demi-dressé	halbaufrecht	semi-erectos	Daliak, Dalkeith	2
adpressed	appliqué	anliegend	tumbados		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>26. C</b> (+)	<b>Leaf: level of <u>formononetin</u> before start of flowering (percentage dry matter)</b>	<b>Feuille: niveau de <u>formononétine</u> avant le début de la floraison (pourcentage de matière sèche)</b>	<b>Blatt: Anteil an <u>Formononetin</u> vor Beginn der Blüte (Prozentsatz von Trockensubstanz)</b>	<b>Hoja: nivel de <u>formononetín</u> antes del comienzo de la floración (porcentaje de materia seca)</b>		
	very low	très bas	sehr niedrig	muy bajo	Dalkeith, Denmark	1
	low	bas	niedrig	bajo	Trikkala	3
	medium	moyen	mittel	medio	Enfield	5
	high	haut	hoch	alto	Geraldton	7
	very high	très haut	sehr hoch	muy alto	Dinninup	9
<b>27. C</b> (+)	<b>Leaf: level of <u>genistein</u> before start of flowering (as for 26)</b>	<b>Feuille: niveau de <u>genistéine</u> avant le début de la floraison (comme pour 26)</b>	<b>Blatt: Anteil an <u>Genistein</u> vor Beginn der Blüte (wie unter 26)</b>	<b>Hoja: nivel de <u>genisteína</u> antes del comienzo de la floración (como para 26)</b>		
	very low	très bas	sehr niedrig	muy bajo	Uniwager	1
	low	bas	niedrig	bajo		3
	medium	moyen	mittel	medio	Dalkeith, Mt Barker	5
	high	haut	hoch	alto	Esperance	7
	very high	très haut	sehr hoch	muy alto	Clare, Gosse, Nuba	9
<b>28. C</b> (+)	<b>Leaf: level of <u>biochanin A</u> before the start of flowering (as for 26)</b>	<b>Feuille: niveau de <u>biochanine A</u> avant le début de la floraison (comme pour 26)</b>	<b>Blatt: Anteil an <u>Biochanin A</u> vor Beginn der Blüte (wie unter 26)</b>	<b>Hoja: nivel de <u>biocanina A</u> antes del comienzo de la floración (como para 26)</b>		
	very low	très bas	sehr niedrig	muy bajo	Dalkeith	1
	low	bas	niedrig	bajo	Clare	3
	medium	moyen	mittel	medio	Yarloop	5
	high	haut	hoch	alto	Dinninup, Dwalganup	7
	very high	très haut	sehr hoch	muy alto	Bacchus Marsh, Seaton Park	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>29. A</b> <b>(*) VS</b>	<b>Stipules: degree of anthocyanin coloration (in shaded part of canopy)</b>	<b>Stipules: degré de coloration anthocyanique (dans la partie ombrée du feuillage)</b>	<b>Nebenblätter: Stärke der Anthocyanfärbung (im beschatteten Teil des Laubes)</b>	<b>Estípulas: grado de pigmentación antocianica (en la parte sombreada del canopeo)</b>		
	absent or very weak	nul ou très faible	fehlend oder sehr gering	ausente o muy débil	Junee	1
	weak	faible	gering	débil	Dalkeith, Goulburn	3
	medium	moyen	mittel	medio	Denmark, York	5
	strong	fort	stark	fuerte	Daliak, Woogenellup	7
	very strong	très fort	sehr stark	muy fuerte	Yarloop	9
<b>30. A</b> <b>(*) MS</b>	<b>Time of start of flowering</b>	<b>Époque de début de la floraison</b>	<b>Zeitpunkt des Blühbeginns</b>	<b>Época de comienzo de la floración</b>		
	very early	très précoce	sehr früh	muy temprana	Nungarin	1
	early	précoce	früh	temprana	Dalkeith	3
	medium	moyenne	mittel	media	Riverina, York	5
	late	tardive	spät	tardía	Goulburn, Mt Barker	7
	very late	très tardive	sehr spät	muy tardía	Tallarook	9
<b>31. A</b> <b>MS</b>	<b>Inflorescence: predominant number of florets</b>	<b>Inflorescence: nombre prédominant de fleurs</b>	<b>Blütenstand: überwiegende Anzahl Blüten</b>	<b>Inflorescencia: número predominante de flores</b>		
	less than three	moins de trois	weniger als drei	menos de tres		1
	three	trois	drei	tres		2
	four	quatre	vier	cuatro		3
	five	cinq	fünf	cinco		4
	more than five	plus de cinq	mehr als fünf	más de cinco		5
<b>32. A</b> <b>(*) VS</b>	<b>Calyx tube: hue</b>	<b>Tube du calice: teinte</b>	<b>Kelchhöhle: Färbung</b>	<b>Tubo del cáliz: matiz</b>		
	absent	absente	fehlend	ausente	Denmark, Junee	1
	present	présente	vorhanden	presente	Dinninup, Mt Barker	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>33. A</b> <b>(*) VS</b>	<b>Calyx tube: color of hue</b>	<b>Tube du calice: couleur de la teinte</b>	<b>Kelchhöhle: Ton der Färbung</b>	<b>Tubo del cáliz: color de la matiz</b>		
	pink	rose	rosa	rosa		1
	pinkish red	rouge rosâtre	rosarot	rojo rosa	Dwalganup, Goulburn	2
	brownish pink	rose brunâtre	bräunlich rosa	rosa parduzco	Riverina, Yarloop	3
	red	rouge	rot	rojo		4
	purplish red	rouge pourpre	purpurrot	rojo púrpura	Mt Barker, Nungarin, York	5
	purple	pourpre	purpur	púrpura	Daliak	6
	brownish purple	pourpre brunâtre	bräunlich purpur	púrpura parduzco		7
	brown	brune	braun	marrón		8
<b>34. A</b> <b>(*) VS</b>	<b>Calyx tube: distribution of coloration</b>	<b>Tube du calice: distribution de la pigmentation</b>	<b>Kelchhöhle: Verteilung der Färbung</b>	<b>Tubo del cáliz: distribución de la pigmentación</b>		
	on upper quarter of tube	sur le quart supérieur du calice	auf dem oberen Viertel des Kelches	en el cuarto superior del cáliz		1
	on upper half of tube	sur la moitié du calice	auf der oberen Hälfte des Kelches	en la mitad superior del cáliz	Goulburn	2
	on upper three-quarters of tube	sur les trois quarts supérieurs du calice	auf dem oberen Dreiviertel des Kelches	en los tres cuartos superiores del cáliz	Mt Barker, Nungarin, York	3
	on entire tube	sur tout le calice	auf dem ganzen Kelch	en todo el cáliz	Daliak	4

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>35. A VS</b>	<b>Peduncle: degree of hairiness</b>	<b>Pédoncule: degré de pilosité</b>	<b>Blütenstandstiel: Ausprägung der Behaarung</b>	<b>Pedúnculo: grado de la pilosidad</b>		
	absent or very weak	absent ou très faible	fehlend oder sehr gering	ausente o muy débil	Denmark	1
	weak	faible	gering	débil	Clare, Goulburn, Junee	3
	medium	moyen	mittel	media	Daliak, Woogenellup, York	5
	strong	fort	stark	fuerte	Dalkeith, Nuba, Seaton Park	7
	very strong	très fort	sehr stark	muy fuerte	Dinninup	9
<b>36. A (* VS)</b>	<b>Stem (runner): degree of hairiness (internode between the 3<sup>rd</sup> and 4<sup>th</sup> node on the longest primary branch)</b>	<b>Tige: degré de pilosité (entrenœud entre le 3<sup>ème</sup> et 4<sup>ème</sup> nœud de la plus longue branche principale)</b>	<b>Ausläufer: Ausprägung der Behaarung (Internodium zwischen dem 3. und 4. Knoten des längsten Hauptzweiges)</b>	<b>Tallo (estolón): grado de la pilosidad (en el entrenudo entre el 3<sup>er</sup> y 4<sup>o</sup> nudo de la ramificación más larga)</b>		
	absent or very weak	nul ou très faible	fehlend oder sehr gering	ausente o muy débil	Denmark, Gosse, Goulburn, Riverina	1
	weak	faible	gering	débil	Junee	3
	medium	moyen	mittel	media	Daliak, Leura, Nuba, York	5
	strong	fort	stark	fuerte	Dalkeith, Nungarin, Seaton Park	7
	very strong	très fort	sehr stark	muy fuerte		9
<b>37. A VS</b>	<b>Stem (runner): attitude of hairs</b>	<b>Tige: port des poils</b>	<b>Ausläufer: Stellung der Haare</b>	<b>Tallo (estolón): porte de los pelos</b>		
	erect	dressé	aufrecht	erecto	Bacchus Marsh, Geraldton, Mt Barker	1
	semi-erect	demi-dressé	halbaufrecht	semi-erecto	Dalkeith	2
	adpressed	appliqué	anliegend	alineado		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>38. B VG</b>	<b>Burr: size</b>	<b>Fruit: taille</b>	<b>Frucht: Größe</b>	<b>Fruto: tamaño</b>		
	very small	très petit	sehr klein	muy pequeño	Daliak, Goulburn	1
	small	petit	klein	pequeño	Geraldton	3
	medium	moyen	mittel	medio	Denmark, Junee	5
	large	grand	groß	grande	Clare	7
	very large	très grand	sehr groß	muy grande	Dalkeith	9
<b>39. B VG</b>	<b>Burr: degree of burial</b>	<b>Fruit: degré d'enfouissement</b>	<b>Frucht: Stärke der Bedeckung mit Erde</b>	<b>Fruto: grado de enterramiento</b>		
	absent or very weak	nul ou très faible	fehlend oder sehr gering	ausente o muy débil	Clare, Nuba, Rosdale	1
	weak	faible	gering	débil	Mt Barker, Woogenellup	3
	medium	moyen	mittel	media	Denmark, Gosse, Goulburn	5
	strong	fort	stark	fuerte	Daliak, Geraldton, Seaton Park	7
	very strong	très fort	sehr stark	muy fuerte	Dalkeith	9
<b>40. B VG</b>	<b>Fruit: predominant number of seeds</b>	<b>Fruit: nombre prédominant des semences</b>	<b>Frucht: überwiegende Anzahl Samen</b>	<b>Fruto: número predominante de semillas</b>		
	less than three	moins de trois	weniger als drei	menos de tres	Gosse	1
	three	trois	drei	tres	Dalkeith	2
	four	quatre	vier	cuatro	Denmark, Goulburn	3
	more than four	plus que quatre	mehr als vier	más que cuatro		4

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>41. B</b> (* ) VG	<b>Seed: color (fresh mature seed)</b>	<b>Semence: couleur (semence mûre fraîche)</b>	<b>Samen: Farbe (frischer reifer Samen)</b>	<b>Semilla: color (semilla madura fresca)</b>		
	white	blanc	weiß	blanca		1
	cream	crème	cremefarben	crema	Gosse, Riverina, Trikkala	2
	light brown	brun clair	hellbraun	marrón clara		3
	purple	pourpre	purpur	púrpura		4
	purplish black	noir pourprâtre	purpurschwarz	negra púrpura	Clare, Mt Barker	5
	black	noire	schwarz	negra	Seaton Park	6
<b>42. C</b>	<b>Seed: weight of 1000 seeds</b>	<b>Semence: poids de 1000 grains</b>	<b>Samen: Tausend-korngewicht</b>	<b>Semilla: peso de 1000 semillas</b>		
	very low	très faible	sehr niedrig	muy bajo	Goulburn	1
	low	faible	niedrig	bajo	Daliak	3
	medium	moyen	mittel	medio	Seaton Park	5
	high	élevé	hoch	alto	Dalkeith, Gosse	7
	very high	très élevé	sehr hoch	muy alto		9
<b>43. C</b> (* ) (+)	<b>Seed: hard seed breakdown over four months</b>	<b>Semence: évolution de la proportion de semences dures après quatre mois</b>	<b>Samen: Brechen von hartem Samen während vier Monaten</b>	<b>Semilla: ruptura de semillas duras durante cuatro meses</b>		
	very slow	très lent	sehr langsam	muy lenta	Geraldton, Northam	1
	slow	lent	langsam	lenta	Dalkeith, Nungarin, York	3
	medium	moyen	mittel	media	June, Seaton Park	5
	fast	rapide	schnell	rápida	Gosse, Riverina	7
	very fast	très rapide	sehr schnell	muy rápida	Mt Barker, Woogenellup	9

VIII. Explanation on the Table of Characteristics

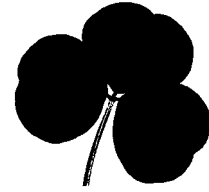
Ad. 4: Leaflet: general shape



1  
triangular



2  
triangular to rounded



3  
rounded

Ad. 7, 10, 16: Leaflet: pattern of mark

Only for varieties with arms: Leaflet: width of arms (characteristic 7)



3  
narrow



5  
medium



7  
broad

Only for varieties with bands: Leaflet: width of bands (characteristic 10)



3  
narrow



5  
medium



7  
broad

Only for varieties with crescent: Leaflet: base of crescent (characteristic 16)



1  
(Type C1)



2  
(Type C2)



3  
(Type C3)



4  
(Type C4)

Ad. 26 to 28: Leaf: level of isoflavones (formononetin, genistein, biochanin A) before start of flowering (percentage dry matters)

Estimating levels of isoflavones. The method is essentially as documented in Francis and Millington (1965). In summary, the procedure is as follows:

Samples

Fresh leaf samples are taken from healthy, recently expanded leaves prior to flowering. Twelve leaf discs per variety are sampled for chemical analysis. A duplicate sample of 12 leaf discs is taken for dry weight calculations.

Extraction of isoflavones

Leaf samples for chemical analysis are macerated in test tubes. After leaving for 15 minutes to allow hydrolysis of bound isoflavones, 1 mL of ethanol (commercial grade, absolute) is added. Samples are placed in a shaking water bath at 60° C for 10 minutes and the extract decanted into clean test tubes. The extraction procedure is repeated on the sample residue to extract any further isoflavones. This solution is added to the initial decanted solution and the total volume made up to 2 mL if necessary with ethanol. To concentrate the sample, a 0.5 mL sub-sample is pipetted into small test tubes and placed in a 40°C oven until all ethanol has evaporated. The sub-sample is then re-dissolved in 0.2 mL of commercial grade ethanol.

Chromatography

An aliquot of 5-µL from each sample is spotted onto Silica gel 60 F<sub>254</sub> thin layer chromatography plates. Standard solutions containing known concentrations of the three isoflavones are also spotted onto each plate. Chromatography is then conducted in a 90:10 solution of chloroform: methanol. Intensity of isoflavone bands is measured under UV light at 254 nm by comparison with the intensity of standard solutions.

### Dry weight samples

The duplicate samples taken for dry weight calculations are dried for 48 hours at 60° C and then weighed.

### Calculations

The level of each isoflavone is calculated as a percentage of dry weight.

### Ad. 43: Seed: hard seed breakdown over four months

#### Seed samples

Fully formed burrs should be obtained from recently senesced plants. Seed production should have proceeded under adequate but not excessively prolonged irrigation or rainfall. Seeds are gently rubbed out of burrs with care taken not to scratch the seed surface.

#### Laboratory procedure

Four hundred seeds of each sample are wetted with water and placed in a 15° C cabinet for 48 hours. Germinated seeds are counted and discarded. The remaining hard-seeds are used for determining rate of breakdown. They are placed in a cabinet fluctuating between 15° C and 60° C over a 24 hour period for 4 months. Samples are then wetted with water and placed in a 15° C cabinet for 48 hours. Germinated seeds are counted. The proportion of hard seeds remaining are calculated as a percentage of the number of hard seeds in the initial sample.



IX. Literature

Dear, B.S. and Sandral, G.A. (1997). Subterranean clover in NSW – identification and use. Agfact P2.5.16, (2<sup>nd</sup> edition), NSW Agriculture, pp. 36.

Francis, C.M. and Millington, A.J. (1965). Varietal variation in the isoflavone content of subterranean clover: its estimation by a microtechnique. *Aust. J. Agric. Res.* 16: 557-564.

Nichols, P.G.H., Collins, W.J. and Barbetti, M.J. (1996). Registered cultivars of subterranean clover - their characteristics, origin and identification. *Agriculture Western Australia Bulletin* No. 4327, pp. 61.

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>			
1.1	Species	<i>Trifolium subterraneum</i>	
		SUBTERRANEAN CLOVER	
1.2	Subspecies	<i>subterraneum</i>	[ ]
		<i>yanninicum</i>	[ ]
		<i>brachycalycinum</i>	[ ]
		other (specify)	[ ]
		.....	
2.	Applicant (Name and address)		
3.	Proposed denomination or breeder's reference		

4. Information on origin, maintenance and reproduction of 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 state of expression which best corresponds).

Characteristics	Example Varieties	Note
<b>5.1 Leaflet: pattern of mark (6)</b>		
a pair of arms only	Yarloop	1[ ]
a single transverse band only	Nungarin	2[ ]
a single, crescent-shaped central mark only	Mt Barker	3[ ]
a pair of arms and a crescent	Seaton Park	4[ ]
<b>5.2 Stipules: degree of anthocyanin coloration (in shaded part of canopy) (29)</b>		
absent or very weak	Junea	1[ ]
weak	Dalkeith, Goulburn	3[ ]
medium	Denmark, York	5[ ]
strong	Daliak, Woogenellup	7[ ]
very strong	Yarloop	9[ ]
<b>5.3 Time of start of flowering (30)</b>		
very early	Nungarin	1[ ]
early	Dalkeith	3[ ]
medium	Riverina, York	5[ ]
late	Goulburn, Mt Barker	7[ ]
very late	Tallarook	9[ ]

Characteristics	Example Varieties	Note
<b>5.4 Calyx tube: distribution of coloration (34)</b>		
on upper quarter of tube		1[ ]
on upper half of tube	Goulburn	2[ ]
on upper three-quarters of tube	Mt Barker, Nungarin, York	3[ ]
on entire tube	Daliak	4[ ]
<b>5.5 Stem (runner): degree of hairiness (internode between the 3<sup>rd</sup> and (36) 4<sup>th</sup> node on the longest primary branch)</b>		
absent or very weak	Denmark, Gosse, Goulburn, Riverina	1[ ]
weak	Junee	3[ ]
medium	Daliak, Leura, Nuba, York	5[ ]
strong	Dalkeith, Nungarin, Seaton Park	7[ ]
very strong		9[ ]
<b>5.6 Seed: hard seed breakdown over four months (43)</b>		
very slow	Geraldton, Northam	1[ ]
slow	Dalkeith, Nungarin, York	3[ ]
medium	Junee, Seaton Park	5[ ]
fast	Gosse, Riverina	7[ ]
very fast	Mt Barker, Woogenellup	9[ ]

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.

7. Additional information which may help to distinguish the variety

7.1 Resistance to pests and diseases

7.2 Special conditions for the examination of the variety

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

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