



**EUROPEAN UNION**

**COMMUNITY PLANT VARIETY OFFICE**

**PROTOCOL FOR DISTINCTNESS, UNIFORMITY AND STABILITY TESTS**

*Vicia faba* L. var. *major* Harz

**BROAD BEAN**

UPOV Species Code: VICIA\_FAB\_MAJ

**Adopted on 25/03/2004**

## **I SUBJECT OF THE PROTOCOL**

The protocol describes the technical procedures to be followed in order to meet the Council Regulation 2100/94 on Community Plant Variety Rights. The technical procedures have been agreed by the Administrative Council and are based on general UPOV Document TG/1/3 and UPOV Guideline TG/206/1 dated 09/04/2003 for the conduct of tests for Distinctness, Uniformity and Stability. This protocol applies to varieties of Broad Bean (*Vicia faba* L. var. *major Harz*).

## **II SUBMISSION OF SEED AND OTHER PLANT MATERIAL**

1. The Community Plant Variety Office (CPVO) is responsible for informing the applicant of

- the closing date for the receipt of plant material;
- the minimum amount and quality of plant material required;
- the examination office to which material is to be sent.

A sub-sample of the material submitted for test will be held in the variety collection as the definitive sample of the candidate variety.

The applicant is responsible for ensuring compliance with any customs and plant health requirements.

2. Final dates for receipt of documentation and material by the Examination Office

The final dates for receipt of requests, technical questionnaires and the final date or submission period for plant material will be decided by the CPVO and each Examination Office chosen.

The Examination Office is responsible for immediately acknowledging the receipt of requests for testing, and technical questionnaires. Immediately after the closing date for the receipt of plant material the Examination Office should inform the CPVO whether acceptable plant material has been received or not. However if unsatisfactory plant material is submitted the CPVO should be informed as soon as possible.

3. Plant material requirements

The final dates for request for technical examination and sending of Technical Questionnaire by the CPVO as well as submission date of plant material by the applicant can be found in the S2 supplement of the CPVO Official Gazette and the CPVO website ([www.cpvo.europa.eu](http://www.cpvo.europa.eu)).

Quality of seed: .....Should not be less than the standards laid down for certified seed in Annex 2 of EC Directive 2002/55.

Quality of plants: .....Should not be less than the standards laid down for plants in EC Directive 92/33 and implementing measures.

Seed Treatment:.....The plant material must not have undergone any treatment unless the CPVO and the examination office allow or request such treatment. If it has been treated, full details of the treatment must be given.

Special requirements: .....-

Labelling of sample:.....- Species

- File number of the application allocated by the CPVO
- Breeder's reference
- Examination reference (if known)
- Name of applicant
- The phrase "On request of the CPVO"
- In the case of a split sample, the quantity of seed being submitted.

### **III CONDUCT OF TESTS**

#### 1. Variety collection

A variety collection will be maintained for the purpose of establishing distinctness of the candidate varieties in test. A variety collection may contain both living material and descriptive information. A variety will be included in a variety collection only if plant material is available to make a technical examination.

Pursuant to Article 7 of Council Regulation No. 2100/94, the basis for a collection should be the following:

- varieties listed or protected at the EU level or at least in one of the EEA Member States;
- varieties protected in other UPOV Member States;
- any other variety in common knowledge.

The composition of the variety collection in each Examination Office depends on the environmental conditions in which the Examination Office is located.

Variety collections will be held under conditions which ensure the long term maintenance of each accession. It is the responsibility of Examination Offices to replace reference material which has deteriorated or become depleted. Replacement material can only be introduced if appropriate tests confirm conformity with the existing reference material. If any difficulties arise for the replacement of reference material Examination Offices must inform the CPVO. If authentic plant material of a variety cannot be supplied to an Examination Office the variety will be removed from the variety collection.

2. Material to be examined

Candidate varieties will be directly compared with other candidates for Community plant variety rights tested at the same Examination Office, and with appropriate varieties in the variety collection. When necessary an Examination Office may also include other candidates and varieties. Examination Offices should therefore make efforts to co-ordinate the work with other Offices involved in DUS testing of broad bean/field bean. There should be at least an exchange of technical questionnaires for each candidate variety, and during the test period, Examination Offices should notify each other and the CPVO of candidate varieties which are likely to present problems in establishing distinctness. In order to solve particular problems Examination Offices may exchange plant material.

3. Characteristics to be used

The characteristics to be used in DUS tests and preparation of descriptions shall be those referred to in the Annex 2. All the characteristics shall be used, providing that observation of a characteristic is not rendered impossible by the expression of any other characteristic, or the expression of a characteristic is prevented by the environmental conditions under which the test is conducted. In the latter case, the CPVO should be informed. In addition the existence of some other regulation e.g. plant health, may make the observation of the characteristic impossible.

The Administrative Council empowers the President, in accordance with Article 23 of Commission Regulation N° 1239/95, to insert additional characteristics and their expressions in respect of a variety.

4. Grouping of varieties

The varieties and candidates to be compared will 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 and which in their various states of expression are fairly evenly distributed throughout the collection. In the case of continuous grouping characteristics overlapping states of expression between adjacent groups is required to reduce the risks of incorrect allocation of candidates to groups. The characteristics used for grouping are the following:

- (a) Plant: growth type (characteristic 2)
- (b) Wing: melanin spot (characteristic 16)
- (c) Dry seed: colour of testa (characteristic 31)
- (d) Dry seed: pigmentation of hilum (characteristic 32)

5. Trial designs and growing conditions

The minimum duration of tests will normally be two independent growing cycles. Tests will be carried out under conditions ensuring normal growth. The size of the plots will be such that plants or parts of plants may be removed for measuring and counting without prejudice to the observations which must be made up to the end of the growing period.

The test design is as follows

As a minimum, each test should include a total of 160 plants which should be divided between two or more replicates.

All observations determined by measurements or counting should be made on 40 plants or parts of 40 plants.

6. Special tests

In accordance with Article 83(3) of Council Regulation No. 2100/94 an applicant may claim either in the Technical Questionnaire or during the test that a candidate has a characteristic which would be helpful in establishing distinctness. If such a claim is made and is supported by reliable technical data, a special test may be undertaken providing that a technically acceptable test procedure can be devised.

Special tests will be undertaken, with the agreement of the President of CPVO, where distinctness is unlikely to be shown using the characters listed in the protocol.

7. Standards for decisions

a) **Distinctness**

A candidate variety will be considered to be distinct if it meets the requirements of Article 7 of Council Regulation No. 2100/94.

b) **Uniformity**

For the assessment of uniformity a population standard of 2% with an acceptance probability of at least 95% should be applied.

Table of maximum numbers of off-types allowed for uniformity standards.

<u>Number of plants</u>	<u>off-types allowed</u>
19-41	2
42-69	3
70-99	4
100-131	5
132-165	6

c) **Stability**

A candidate will be considered to be sufficiently stable when there is no evidence to indicate that it lacks uniformity.

**IV REPORTING OF RESULTS**

After each recording season the results will be summarised and reported to the CPVO in the form of a UPOV model interim report in which any problems will be indicated under the headings distinctness, uniformity and stability. Candidates may meet the DUS standards after two growing periods but in some cases three growing periods may be required. When tests are completed the results will be sent by the Examination Office to the CPVO in the form of a UPOV model final report.

If it is considered that the candidate complies with the DUS standards, the final report will be accompanied by a variety description in the format recommended by UPOV. If not the reasons for failure and a summary of the test results will be included with the final report.

The CPVO must receive interim reports and final reports by the date agreed between the CPVO and the examination office.

Interim reports and final examination reports shall be signed by the responsible member of the staff of the Examination Office and shall expressly acknowledge the exclusive rights of disposal of CPVO.

## **V LIAISON WITH THE APPLICANT**

If problems arise during the course of the test the CPVO should be informed immediately so that the information can be passed on to the applicant. Subject to prior agreement, the applicant may be directly informed at the same time as the CPVO particularly if a visit to the trial is advisable.

The interim report as well as the final report shall be sent by the Examination Office to the CPVO.

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## ANNEXES TO FOLLOW

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## **ANNEX II**

Technical Questionnaire



## ANNEX I

### TABLE OF CHARACTERISTICS TO BE USED IN DUS-TEST AND PREPARATION OF DESCRIPTIONS

CPVO N°	UPOV N°	Stage, Method	Characteristics	Examples	Note
1. (+)	1.	00	<b>Seed: tannin</b>		
			absent	Driemaal Wit	1
			present	Trio	9
2.  G	2.	65-67	<b>Plant: growth type</b>		
			determinate	Samson, Smerf	1
			indeterminate	Driemaal Wit	2
3.	3.	60-69	<b>Plant: height</b>		
			very short	The Sutton	1
			short	Arbo, Reina Mora	3
			medium	Aquadulce Claudia	5
			tall	Dreadnought	7
			very tall	Imperial White Windsor	9
4.	4.	60-69	<b>Plant: number of stems (including tillers more than half the length of the main stem)</b>		
			few	The Sutton	3
			medium	Albinette, Arbo	5
			many	Reina Blanca	7
5.	5.	60-69	<b>Stem: number of nodes up to and including first flowering node</b>		
			few	Driemaal Wit, Metissa	3
			medium	Futura	5
			many		7
6.	6.	39-69	<b>Stem: anthocyanin coloration</b>		
			absent	Driemaal Wit, Metissa	1
			present	Futura	9

CPVO N°	UPOV N°	Stage, Method	Characteristics	Examples	Note	
7.	7.	39-69	<b>Foliage: greyish hue of green colour</b>			
			(a)	absent	Metissa	1
			present	Osnaweiss	9	
8.	8.	39-69	<b>Foliage: intensity of green colour</b>			
			(a)	light	Driemaal Wit	3
				medium	Express, Futura	5
			dark		7	
9.	9.	62-65	<b>Leaflet: length (basal pair of leaflet at secondary node)</b>			
			(b)	short	Metissa	3
				medium	Supraguadulce Tézier, Futura	5
			long	Lange Hangers, Osnabrücker Markt	7	
10.	10.	62-65	<b>Leaflet width (basal pair of leaflet at secondary node)</b>			
			(b)	narrow	The Sutton	3
				medium	Optica	5
			broad	Osnabrücker Markt	7	
11.	11.	62-65	<b>Leaflet: position of maximum width (basal pair of leaflet at secondary node)</b>			
			(b)	towards tip		1
				at middle		2
			towards base		3	
12.	12.	70-78	<b>Leaflet: folding (along the main vein, terminal pair of leaflets)</b>			
			(b)	weak	Metissa	3
				medium		5
			strong	Minica	7	

CPVO N°	UPOV N°	Stage, Method	Characteristics	Examples	Note
13.	13.	62-65  (b)	<b>Raceme: number of flowers</b>		
			few	Aquadulce Claudia	3
			medium		5
			many		7
14.	14.60		<b>Time of flowering (50% of the plants with at least one flower)</b>		
			early	Minica, Optica	3
			medium	Futura	5
			late	Osnabrücker Markt	7
15.  (+)	15.	60-65  (b)	<b>Flower: length</b>		
			short	Aquadulce Claudia, The Sutton	3
			medium	Minica	5
			long	Green Windsor	7
16.  G	16.	60-65	<b>Wing: melanin spot</b>		
			absent	Driemaal Wit, Metissa	1
			present	Futura, Trio	9
17.	17.	60-65	<b>Wing: colour of melanin spot</b>		
			greenish yellow	Golda	1
			brown		2
			black	Futura, Trio	3
18.	18.	60-65	<b>Standard: melanin spot</b>		
			absent	Driemaal Wit, Futura	1
			present	Felix	9
19.	19.	60-65	<b>Standard: anthocyanin coloration</b>		
			absent	Driemaal Wit	1
			present		9

CPVO N°	UPOV N°	Stage, Method	Characteristics	Examples	Note	
20.	20	60-65	<b>Standard: extent of anthocyanin coloration</b>			
			(+)	small	The Sutton, Osnabrücker Markt	3
				medium		5
				large		7
21.	21.	70-78	<b>Truss: number of pods</b>			
				few	Aquadulce Claudia, Muchamiel	3
				medium	Metissa	5
				many		7
22.	22.	75-80	<b>Pod: attitude</b>			
			(a)	erect	Optica	1
			(b)	semi-erect	Statissa, The Sutton	3
				horizontal	Trio	5
				semi-pendulous	Express	7
				pendulous	Lange Hangers, Futura	9
23.	23.	80	<b>Pod: length (without beak)</b>			
			(a)	very short	Arbo	1
			(b)	short	Green Windsor, Optica	3
				medium	Driemaal Wit, Red Epicure	5
				long	Dreadnought	7
				very long	Hangdown Grünkernig	9
24.	24.	80	<b>Pod: width (from suture to suture)</b>			
			(a)	narrow	Felix, Minica	3
			(b)	medium	Trio, Express	5
				broad	Con Amore	7
				very broad	Aquadulce Claudia	9

CPVO N°	UPOV N°	Stage, Method	Characteristics	Examples	Note	
25.  (+)	25.	80	<b>Pod: degree of curvature at green shell stage</b>			
			(b)	absent or very weak	Optica	1
				weak	Metissa	3
				medium	Witkiem	5
				strong	Groene Hangers, Futura	7
26.	26.	80	<b>Pod: intensity of green colour</b>			
			(a)	light	Futura	3
			(b)	medium	Driemaal Wit	5
			dark	Statissa	7	
27.	27.	80	<b>Pod: number of ovules (including seeds)</b>			
			(a)	few	White Windsor	3
			(b)	medium	Aquadulce Claudia	5
			many	Imperial Green, Longpod	7	
28.	29.	99	<b>Dry seed: shape of median longitudinal section</b>			
			(c)	narrow elliptic	Metissa	1
				elliptic		2
			broad elliptic	Futura	3	
29.	30.	99	<b>Dry seed: shape of cross section</b>			
			(c)	narrow elliptic	Aquadulce Claudia, Futura	1
				elliptic		2
			broad elliptic		3	
30.  (+)	31.	99	<b>Dry seed: weight</b>			
			(c)	very low	Albinette, Minica	1
				low	Arbo, Felix	3
				medium	The Sutton, Trio	5
				high	Futura, Red Epicure	7
			very high	White Windsor	9	

CPVO N°	UPOV N°	Stage, Method	Characteristics	Examples	Note	
31.	32.	99	<b>Dry seed: colour of testa (immediately after harvest)</b>			
			(c)	beige	Driemaal Wit, Trio	1
				green	Green Windsor	2
				red	Red Epicure	3
				violet	Reina Mora	4
G			black		5	
32	33.	99	<b>Dry seed: pigmentation of hilum</b>			
			(c)	absent	Driemaal Wit	1
G			present	Aquadulce Claudia	9	
33.	34.	80-85	<b>Time of full development of pod (first fully developed pods)</b>			
				early	Express	3
				medium	Driemaal Wit	5
			late	Imperial Green Longpod	7	

## EXPLANATIONS AND METHODS

Characteristics containing the following key in the second column of the Table of Characteristics should be examined as indicated below:

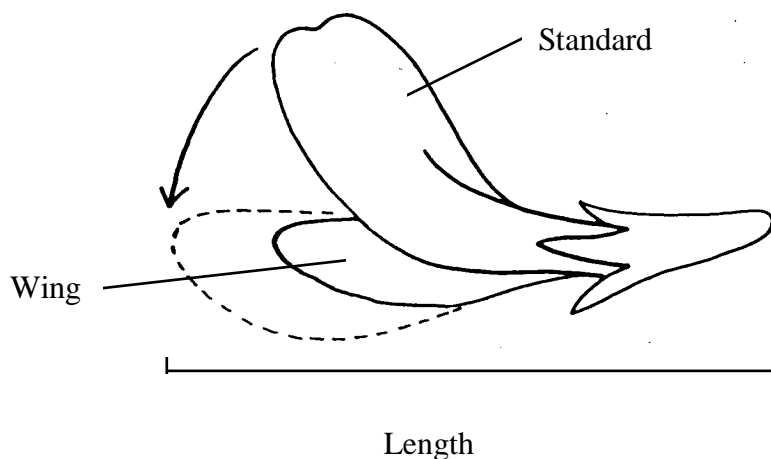
- (a) Foliage and pod: Unless otherwise indicated, all observations on the foliage and pod should be made before green harvest maturity.
- (b) Leaflet, raceme, flower, pod: All measurements of the leaflet, flower and pod should be made at the 2<sup>nd</sup> flowering node.
- (c) Dry seed: All observations on the dry seed should be made on harvested dry seed.

### Ad. 1: Seed: tannin

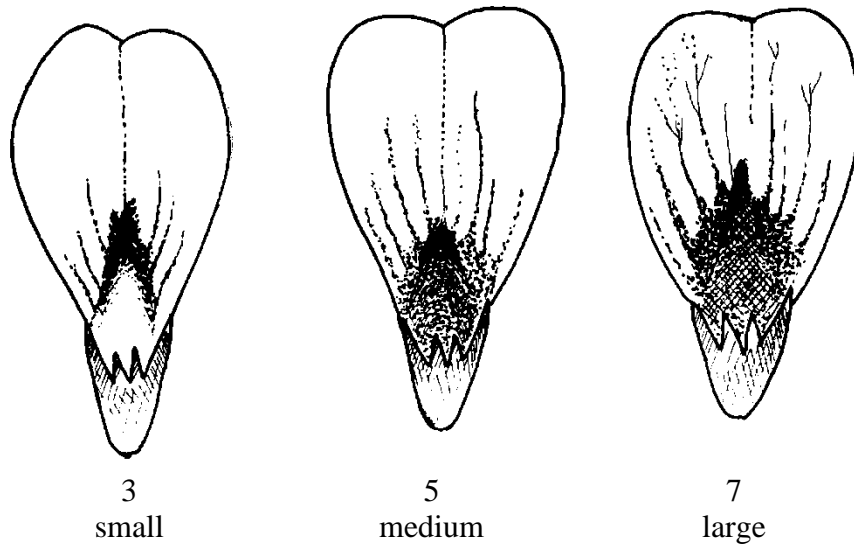
The tannin content of testa correlates with melanin spot on the flower wing. Maintaining both characteristics is necessary, as observations are made at very different stages and different times. The content of tannin should be tested by removing a piece of the testa from the seed and placing 1 or 2 drops of the test reagent upon its inner surface. A bright pink colour will develop within 1 or 2 minutes in the presence of tannins. (Reagents: A = 50% ethanol; B = 1% vanillin in concentrated HCl. A and B mixed 1:1 for use. For the purposes of this test, 'concentrated' is defined as within the range 33-37% weight by volume.)

Seeds that are yellowish grey immediately after harvest will turn brown after ageing if they contain tannin.

### Ad. 15: Flower: length



Ad. 20: Standard: extent of anthocyanin coloration



The observation should be made on the inner side of the Standard.



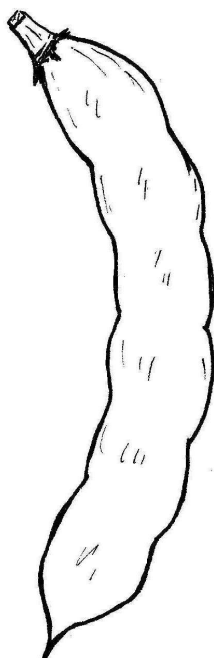
Ad. 25: Pod: degree of curvature at green shell stage



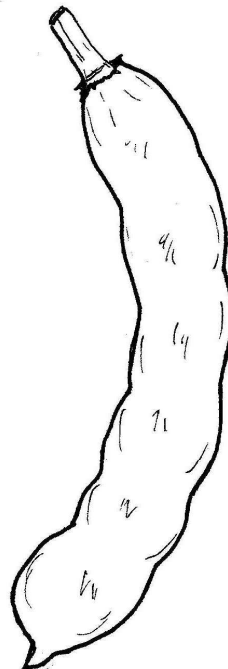
1  
absent or very weak



3  
weak



5  
medium



7  
strong

Ad. 30: Dry seed: weight

The dry seed weight should be measured by weighing the largest seed from the largest pod for each plant sampled.

***Phenological growth stages and BBCH-identification keys of Vicia faba L. (Meier, 1997)***

<b>Code</b>	<b>Description</b>
<b>Principal growth stage 0: Germination</b>	
00	Dry seed
01	Beginning of seed imbibition
02	–
03	Seed imbibition complete
04	–
05	Radicle emerged from seed
06	–
07	Shoot emerged from seed (plumule apparent)
08	Shoot growing towards soil surface
09	Emergence shoot emerges through soil surface
<b>Principal growth stage 1: Leaf development <sup>1</sup></b>	
10	Pair of scale leaves visible (may be eaten or lost)
11	First leaf unfolded
12	2 leaves unfolded
13	3 leaves unfolded
14	4 leaves unfolded
15	5 leaves unfolded
16	6 leaves unfolded
17	7 leaves unfolded
18	8 leaves unfolded
19	9 or more leaves unfolded
<b>Principal growth stage 2: Formation of side shoots</b>	
20	No side shoots
21	Beginning of side shoot development: first side shoot detectable
22	2 side shoots detectable
23	3 side shoots detectable
24	4 side shoots detectable
25	5 side shoots detectable
26	6 side shoots detectable
27	7 side shoots detectable
28	8 side shoots detectable
29	End of side shoot development: 9 or more side shoots detectable
<b>Principal growth stage 3: Stem elongation</b>	
30	Beginning of stem elongation
31	One visibly extended internode <sup>2</sup>
32	2 visibly extended internodes
33	3 visibly extended internodes
34	4 visibly extended internodes
35	5 visibly extended internodes
36	6 visibly extended internodes
37	7 visibly extended internodes
38	8 visibly extended internodes
39	9 or more visibly extended internodes
<b>Principal growth stage 4: -----</b>	

<sup>1</sup> Stem elongation may occur earlier than stage 19; in this case continue with the principal stage 3.

<sup>2</sup> First internode extends from the scale leaf node to the first true leaf node.

Code	Description
<b>Principal growth stage 5: Inflorescence emergence</b>	
50	Flower buds present, still enclosed by leaves
51	First flower buds visible outside leaves
52	–
53	–
54	–
55	First individual flower buds visible outside leaves but still closed
56	–
57	–
58	–
59	First petals visible, many individual flower buds, still closed
<b>Principal growth stage 6: Flowering</b>	
60	First flowers open
61	Flowers open on first raceme
62	–
63	Flowers open 3 racemes per plant
64	–
65	Full flowering: flowers open on 5 racemes per plant
66	–
67	Flowering declining
68	–
69	End of flowering
<b>Principal growth stage 7: Development of fruit</b>	
70	First pods have reached final length (“flat pod”)
71	10% of pods have reached final length
72	20% of pods have reached final length
73	30% of pods have reached final length
74	40% of pods have reached final length
75	50% of pods have reached final length
76	60% of pods have reached final length
77	70% of pods have reached final length
78	80% of pods have reached final length
79	Nearly all pods have reached final length
<b>Principal growth stage 8: Ripening</b>	
80	Beginning of ripening: seed green, filling pod cavity
81	10% of pods ripe, seeds dry and hard
82	20% of pods ripe, seeds dry and hard
83	30% of pods ripe and dark, seeds dry and hard
84	40% of pods ripe and dark, seeds dry and hard
85	50% of pods ripe and dark, seeds dry and hard
86	60% of pods ripe and dark, seeds dry and hard
87	70% of pods ripe and dark, seeds dry and hard
88	80% of pods ripe and dark, seeds dry and hard
89	Fully ripe: nearly all pods dark, seeds dry and hard

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<b>Code</b>	<b>Description</b>
<b>Principal growth stage 9: Senescence</b>	
90	–
91	–
92	–
93	Stems begin to darken
94	–
95	50% of stems brown or black
96	–
97	Plant dead and dry
98	–
99	Harvested product

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## **ANNEX II**

The Technical Questionnaire is available on the CPVO website under the following reference:  
CPVO-TQ/206/1