



EUROPEAN UNION

COMMUNITY PLANT VARIETY OFFICE

PROTOCOL FOR DISTINCTNESS, UNIFORMITY AND STABILITY TESTS

Brassica napus L. emend. Metzg.

RAPE SEED

UPOV Species Code: BRASS_NAP

Adopted on 25/03/2004

I - SUBJECT OF THE PROTOCOL

The protocol describes the technical procedures to be followed in order to meet the requirements of 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/36/6 dated 18th October 1996 for the conduct of tests for Distinctness, Uniformity and Stability. This protocol applies to all varieties of *Brassica napus* L. emend. Metzg.

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 of the Examination Office 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 if no plant material has been received. However, if unsatisfactory plant material is submitted the CPVO should be informed as soon as possible.

3. Seed requirements

Information with respect to closing dates and submission requirements of plant material for the technical examination of varieties can be found on the CPVO web site (www.cpvo.europa.eu) and in the special Issue S2 of the Official Gazette of the Office published yearly at the month of September.

Quality of seed: The minimum requirements for germination capacity, analytical purity and seed health should not be less than the standards laid down in EC Directive 2002/57/EC

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.

Labelling of sample: .. - Species

- File number of the application allocated by the CPVO
- Breeder's reference
- Examination Office reference (if known)
- Name of applicant
- The phrase "On request of the CPVO".

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.
- In case of hybrids, all components in common knowledge must be considered as part of the reference collection.

The composition of the variety collection in each Examination Office depends on the ecological 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 rape seed. There should be at least an exchange of information about candidate varieties 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 table of characteristics. 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 that could be used for grouping are the following (CPVO numbering; G for grouping in table of characteristics)

- a) Seed: erucic acid (characteristic 1)
- b) Leaf: lobes (characteristic 6)
- c) Time of flowering (characteristic 9)

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 cycle.

The test design is as follows:

If not otherwise indicated, each test should include at least 200 plants which should be divided between two or more replicates.

The assessment for the characteristic 'Tendency to form inflorescences' should be carried out on at least 100 plants.

If tests on progenies of unthreshed plants are conducted, these should include at least 40 progenies.

In case of hybrids, components (including the maintainer line) have to be tested and assessed as any other variety.

Unless otherwise stated, all observations for the assessment of distinctness on individual plants should be made on 60 plants or parts of 60 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.

To assess distinctness of hybrids, a pre-screening system on the basis of the parental lines and the formula may be established according to the following recommendations:

- (i) description of parental lines according to the Test Guidelines;
- (ii) check of the originality of the parental lines in comparison with the reference collection, based on the characteristics in the table of characteristics in order to screen the closest inbred lines;

- (iii) check of the originality of the hybrid formula in comparison with those of the hybrids in common knowledge, taking into account the closest inbred lines;
- (iv) assessment of the distinctness at the hybrid level of varieties with a similar formula.

Qualitative characteristics:

In the case of characteristics which show discrete discontinuous states of expression, a difference between two varieties is clear if the respective characteristics have expressions which fall into two different states.

Quantitative characteristics:

Characteristics which show a continuous range of expression from one extreme to the other may be either measured or visually observed.

In the case of visually observed characteristics, a difference between two varieties is clear if the expression of the respective characteristics differs by at least the span of one note, taking into account the variability observed within the varieties.

In the case of observations on individual plants, the combined over years analysis (COYD) should be applied for the assessment of distinctness. The appropriate significance level should be defined according to the recommendations developed by UPOV.

If the significance level or statistical methods proposed are not appropriate the method used should be clearly described.

b) Uniformity

In case of visual observation, uniformity is assessed on the basis of off-types.

In case of measurements, uniformity should be assessed by using COYU or other appropriate statistical methods.

For the assessment of uniformity on the basis of off-types, the number of the aberrant plants or parts of plants should be counted on the total of 200 plants.

For the assessment of uniformity of lines a population standard of 2% with an acceptance probability of at least 95% should be applied. In the case of hybrids, the population standard should be 10% with the same acceptance probability of at least 95%.

In case of progenies of unthreshed plants are observed, the tolerance for uniformity in the progenies should be four off-type progenies in 40 (population standard of 5% with an acceptance probability of at least 95%).

c) **Stability**

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

Seed samples of further submissions included in any test must show the same expression of characteristics as the material originally supplied.

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 cycles but in some cases three growing cycles 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 so that the information can be passed on to the applicant. Subject to prior permanent agreement, the applicant may be directly informed at the same time as the CPVO particularly if a visit to the trial is advisable.

VI - TABLE OF CHARACTERISTICS TO BE USED IN DUS-TEST AND PREPARATION OF DESCRIPTION

CPVO N°	UPOV N°	Characteristics	Stage ¹	Examples ²	Note
1. (+) ³ G	1.	Seed: erucic acid	00		
		absent		Cadoma ; Express	1
		present		Rabelais ; Zeruca	9
2. (+)	2.	Cotyledon: length	15-17		
		short		Sponsor ; Bristol	3
		medium		Star ; Apex	5
		long	Calisto ; -	7	
3. (+)	3.	Cotyledon: width	15-17		
		narrow		Tracia ; Pirola	3
		medium		Dorothy ; Apex	5
		broad	Calida ; Jockey	7	
4.	4.	Leaf: green colour	23-27		
		light		Calida ; Jockey	3
		medium		Star ; Express	5
		dark	- ; Capitol	7	
5.		Leaf: glaucosity	23-27		
		absent			1
		present		9	
6. (+) G	5.	Leaf: lobes	23-27		
		absent		Calida ; Akela	1
		present	Dorothy ; Express	9	

¹ The optimum stage of development is indicated by numbers. Explanations are given in Annex 1 in 'Explanations and Methods'.

² Example varieties, separated by a semicolon, are indicated for spring rape seed before the semicolon, for winter rape seed they follow the semicolon. Example varieties are given as an indication, others may be used.

³ See explanations in Annex 1 in 'Explanations and Methods'

CPVO N°	UPOV N°	Characteristics	Stage ¹	Examples ²	Note	
7.	6.	Leaf: number of lobes (fully developed leaf)	23-27			
		(+)		few	Quantum; -	3
				medium	Dorothy ; Bristol	5
				many	- ; Dante	7
8.	7.	Leaf: dentation of margin	23-27			
		(+)		weak	Cadoma ; Ecuror	3
				medium	NEX160 ; Mohican	5
				strong	Pactol ; Lutin	7
9.	11.	Time of flowering	61-62			
		(+)		very early	Cadoma ; -	1
				early	Corona ; ISLR3	3
				medium	Liaison ; Zenith	5
				late	Arista ; MSL 007 C	7
		G		very late	Astor ; -	9
10.	12.	Flower: colour of petals	62-63			
				white		1
				cream		2
				yellow	Pactol ; Bristol	3
				orange-yellow		4
11.	13.	Flower: length of petals	62-63			
				very short	2500; -	1
				short	MLCP30 ; CS08	3
				medium	Dorothy ; Apex	5
				long	Ester ; Sioux	7
12.	14.	Flower: width of petals	62-63			
				narrow	Calisto ; CS08	3
				medium	Calida ; Pirola	5
				broad	Tracia ; Sioux	7

CPVO N°	UPOV N°	Characteristics	Stage ¹	Examples ²	Note
13.	15.	Production of pollen	62-63		
		absent		MSL 501 C ; CS08	1
		present		Calida ; Apex	9
14. (+)	16.	Plant: height (at full flowering)	64		
		very low		2500 ; Lutin	1
		low		Sponsor ; Prestol	3
		medium		Star ; Tosca	5
		tall		Arista ; Capitol	7
		very tall		Astor ; -	9
15.	17.	Plant: total length including side branches	75-80		
		very short		2500 ; Lutin	1
		short		Cadoma ; Montego	3
		medium		Star ; Boston	5
		long		Arista ; Amber	7
		very long		Astor ; -	9
16. (+)	18.	Siliqua: length (between peduncle and beak)	75-89		
		very short		- ; ISLR3	1
		short		Calida ; Pirola	3
		medium		Dorothy ; Bristol	5
		long		Master ; Mohican	7
17. (+)		Siliqua: width	75-89		
		narrow		Calida ; ISLR3	3
		medium		Calisto ; Apex	5
		broad		Cadoma ; Capitol	7
		very broad		- ; CS11	9

CPVO N°	UPOV N°	Characteristics	Stage ¹	Examples ²	Note	
18.	19.	Silique: length of beak	75-89			
		(+)		very short	- ; ISLR3	1
				short	Calisto ; Dante	3
				medium	Calida ; Apex	5
				long	Liaison ; Tosca	7
				very long	- ; BL643196	9
19.	20.	Silique: length of peduncle	75-89			
		(+)		short	Calisto ; Express	3
				medium	Star ; Apex	5
		long	Sponsor ; Inca	7		
20.	21.	Tendency to form inflorescences in year of sowing for <u>spring</u> sown trials				
		(+)	absent or very weak	Silex	1	
			weak	Mohican	3	
			medium	Tosca	5	
			strong	Zeruca	7	
			very strong	Montego	9	
21.	22.	Tendency to form inflorescences in year of sowing for <u>late summer</u> sown trials				
		(+)	absent or very weak	Silex	1	
			weak	Tanto	3	
			medium	Calida	5	
			strong	MLCP 30	7	
			very strong	Cadoma	9	

ANNEXES TO FOLLOW

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ANNEX II
Technical Questionnaire

ANNEX I

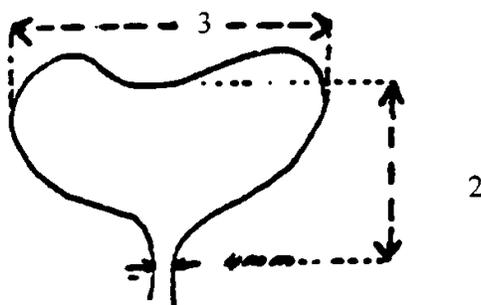
EXPLANATIONS AND METHODS

Ad. 1: Seed: erucic acid

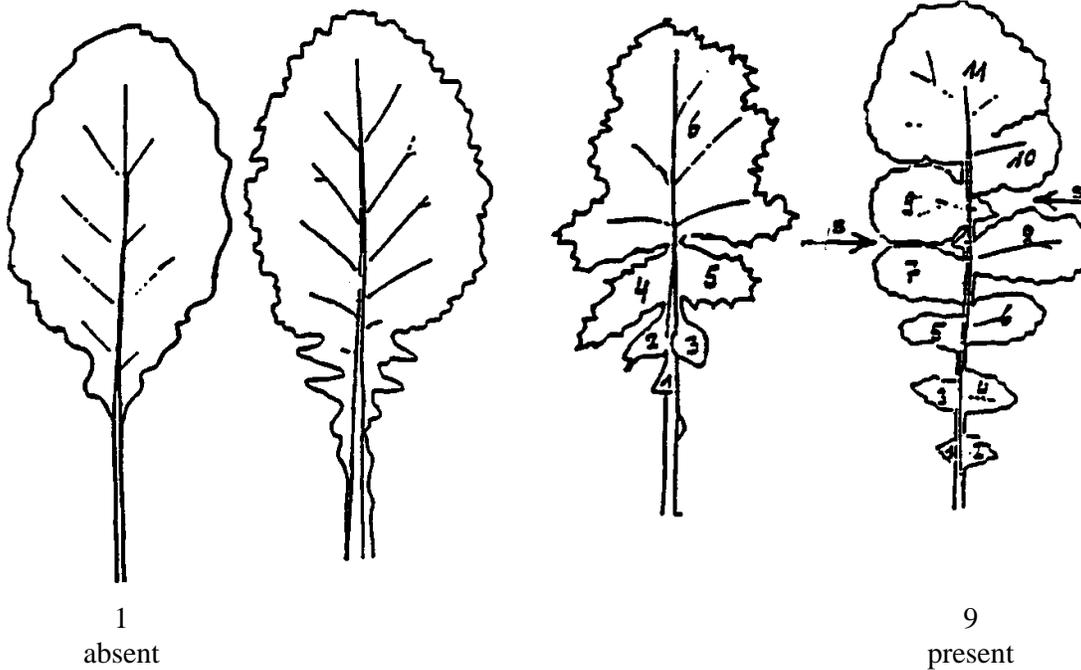
The erucic acid content should be observed on seed sent in by the applicant. It should be expressed as a percentage by mass of methyl esters in accordance with the ISO standard in document 5508, paragraph 6.2.2.1. Seed containing 2% or less of erucic acid will be classified as “absent.”

Ad. 2 + 3: Cotyledon: length (2) and width (3)

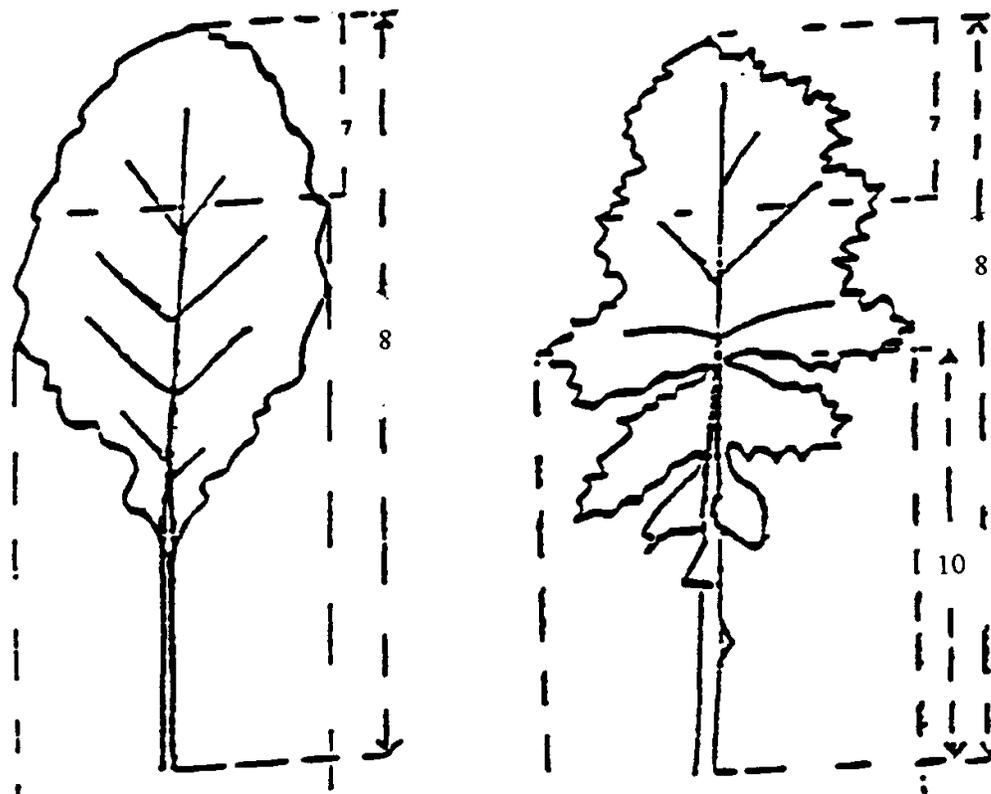
The measurements should be taken in the glasshouse on cotyledons of 40 seedlings. If the two cotyledons differ in size, the biggest one should be measured. The length is defined as distance between the inclination at top of the cotyledon and the point where the width of the petiole is about 4 mm. The width of the cotyledon should be measured at the widest point of the cotyledons.



Ad. 6 + 7: Leaf: presence and number of lobes



Absence or presence of lobing should be observed on the whole plant at rosette stage. Parts of the leaf blade are considered as lobes if their length is at least equivalent to the width of the leaf petiole at their point of attachment and if the upper notch of the blade has at least half the length of the lobe itself. Secondary lobes (s) are not counted.

Ad. 8: Leaf: dentation

7 = part on which the dentation should be recorded (characteristic 7)

Ad. 9: Time of flowering

The observation should be done at least three times per week and more frequently if there is any need to do so. When assessed on individual plants, the date should be calculated--if necessary by interpolation--at which 50% of plants show at least one open flower. When assessed on the plot as a whole, the recommended percentage is 10%.

Ad.14: Plant: height (at full flowering)

The height of the plants should be assessed when all normally developed plants have opened at least one flower.

Ad. 16 - 19: Siliqua

All observations on the siliqua should be recorded in the mid-part of the inflorescence of the main stem.

Ad. 20 + 21: Tendency to form inflorescences in the year of sowing

The tendency to form inflorescences in the year of sowing of winter rape varieties should be recorded in spring sown trials; that of spring rape varieties in late summer sown trials. The observation of the growth stage reached of winter rape varieties should be made in summer when the late spring rape varieties are flowering; that of spring rape varieties in autumn, when their development stagnates.

KEY FOR THE GROWTH STAGES

KEY	GENERAL DESCRIPTION
0	<u>Germination</u>
00	Dry Seed
10	<u>Seedling growth</u>
11	Appearance of cotyledons
13	Cotyledons expanded
15	1 leaf-stage
17	2 leaf-stage
19	3 leaf-stage
20	<u>Rosette</u>
21	4 leaf-stage
22	5 leaf-stage
23	6 leaf-stage
24	7 leaf-stage
25	8 leaf-stage
26	9-11 leaf-stage
27	12 or more leaves are completely developed
30	<u>Stem elongation</u>
31	Distance between cotyledons and vegetation point is more than 5 cm
35	Distance between cotyledons and vegetation point is more than 15 cm
39	Distance between cotyledons and vegetation point is more than 25 cm
50	<u>Bud formation</u>
51	Terminal bud is present, not raised above leaves
53	Terminal bud is raised above level of leaves
57	Pedicels are elongating
59	Buds are yellowing

KEY	GENERAL DESCRIPTION
60	<u>Flower</u>
61	First open bud on terminal raceme
62	Few buds are open on terminal raceme
64	Full flower, lower siliques are elongating
65	Lower siliques are starting to fill, less than 5% of buds are not yet open
67	Seeds in lower siliques are enlarging, all buds are open
70	<u>Silique</u>
71	Seeds in lower siliques are in full size translucent
75	Seeds in lower siliques are green, opaque
79	All seeds of siliques on terminal raceme are dark
80	<u>Maturation</u>
81	Seeds in lower siliques on terminal raceme show brown areas
85	Seeds in upper siliques show brown areas
89	Brown siliques are brittle, stems are dry



germination



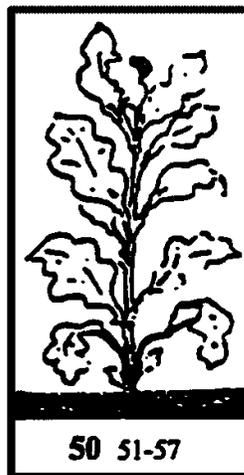
seedling growth



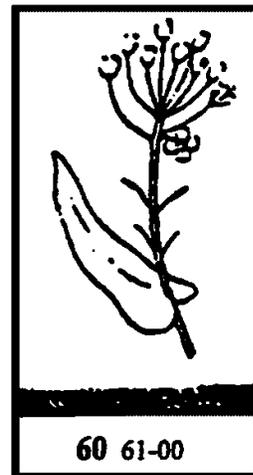
rosette



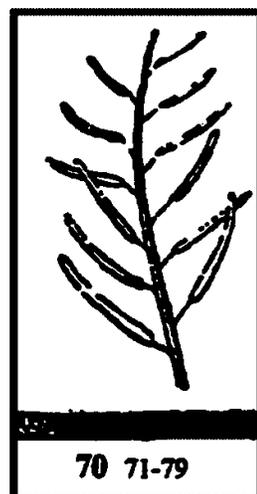
stem elongation



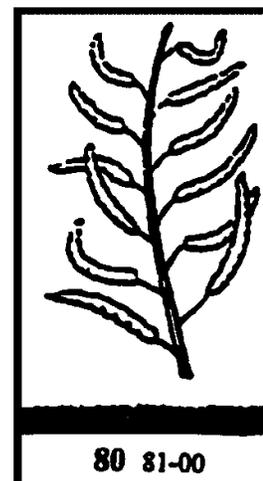
bud formation



flower



silique



maturation

ANNEX II



European Union
Community Plant Variety Office

TECHNICAL QUESTIONNAIRE

to be completed in connection with an application for Community Plant Variety Rights
Please answer all questions. A question without any answer will lead to a non-attribution
of an application date. In cases where a field / question is not applicable, please state so.

1.1 Botanical taxon: Name of the genus, species or sub-species to which the variety belongs and
common name

Brassica napus L. emend. Metzg

RAPE SEED

1.1 Forma *ibernalis*

1.2 Forma *aestiva*

2.1 Applicant(s): Name(s) and address(es), phone and fax number(s), Email address, and where
appropriate name and address of the procedural representative

3.1 Variety denomination

a) Where appropriate proposal for a variety denomination:

b) Provisional designation (breeder's reference):

4 Information on origin, maintenance and reproduction of the variety**4.1 Type of material**

- (a) line use (only for male fertile line)
 - male fertile line as component
 as commercial variety
 - male sterile line
- (b) hybrid
 - male sterile hybrid
 - male fertile hybrid
 - self incompatible hybrid
- (c) other (please indicate)

4.2 Formula (if applicable, for each component in separate sheets, the information according to the following chapter 5 to 7 to be added)Single hybrid

- Denomination or breeder's reference of female parental line
- Denomination or breeder's reference of male parental line

Three-way hybrid

Denomination or breeder's reference of:

- single hybrid used
- female parental line of the single hybrid
- male parental line of the single hybrid
- female parent of the three-way hybrid
- male parental line of the three-way hybrid

NB: In case of use of male sterility system, indicate the name of the maintainer line of the female parental line

In case of use of self-incompatibility system, indicate if applicable the name of the self-compatible lines

4.3 Origin

- (a) Seedling (indicate parent varieties) []

- (b) Mutation (indicate parent variety)..... []

- (c) Discovery (indicate where, when
and how the variety has been developed): []

- (d) Other (please specify) []

4.4 Method of propagation

- (a) Cuttings..... []
- (b) *In vitro* propagation..... []
- (c) Seed..... []
- (d) Other (please specify): []

4.5 Other information:

In the case of seed propagated varieties: method of production:

- (a) Self-pollinated..... []
- (b) Cross-pollinated (please give details) []

- (c) Hybrid (please give details) []

4.6 Geographical origin of the variety: the region and the country in which the variety was bred or discovered and developed

4.7 Shall the information on data relating to components of hybrid varieties including data related to their cultivation be treated as confidential?

YES NO

If yes, please give this information on the attached form for confidential information.

If no, please give information on data relating to components of hybrid varieties including data related to their cultivation:

Breeding scheme (indicate female component first)

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

	Characteristics	Example varieties	Note
5.1 (1)	Seed: erucic acid		
	absent	Cadoma; Express	1 []
	present	Rabelais; Zeruca	9 []
5.2 (6)	Leaf: lobes		
	absent	Calida; Akela	1 []
	present	Dorothy; Express	9 []
5.3 (9)	Time of flowering (quote mean date of flowering of variety as well as of two well-known comparable varieties)		
		
		
		

7.2 Resistance to pests and diseases

7.3 Other information

YES, please specify

NO

8. GMO-information required

The variety represents a Genetically Modified Organism within the meaning of Article 2(2) of Council Directive EC/2001/18 of 12/03/2001.

YES NO

If yes, please add a copy of the written attestation of the responsible authorities stating that a technical examination of the variety under Articles 55 and 56 of the Basic Regulation does not pose risks to the environment according to the norms of the above-mentioned Directive.

9. Information on plant material to be examined

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) | <input type="checkbox"/> Yes | <input type="checkbox"/> No |
| (b) Chemical treatment (e.g. growth retardant or pesticide) | <input type="checkbox"/> Yes | <input type="checkbox"/> No |
| (c) Tissue culture | <input type="checkbox"/> Yes | <input type="checkbox"/> No |
| (d) Other factors | <input type="checkbox"/> Yes | <input type="checkbox"/> No |

Please provide details of where you have indicated “Yes”:

I/we hereby declare that to the best of my/our knowledge the information given in this form is complete and correct.

Date

Signature

Name

[End of document]