



**European Union
Community Plant Variety Office**

PROTOCOL FOR DISTINCTNESS, UNIFORMITY AND STABILITY TESTS

Eustoma grandiflorum (Raf.) Shinnery

EUSTOMA (LISIANTHUS)

UPOV Species Code: EUSTO_GRA

Adopted on 1st December 2005

I - SUBJECT OF THE PROTOCOL

The protocol describes the technical procedures to be followed in order to meet the requirement 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/197/1 dated 17/04/2002 for the conduct of tests for Distinctness, Uniformity and Stability. This protocol applies to all varieties of *Eustoma gradiflorum* (Raf.) Shinnars (synonym: *Eustoma russellianum* (Hook.) G. Don) of the family *Gentianaceae*.

II - SUBMISSION OF 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.

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. If no or unsatisfactory plant material is submitted the CPVO should be informed as soon as possible.

3. Plant material 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 website (www.cpvo.europa.eu) and in the special Issue S2 of the Official Gazette of the Office published yearly in the month of September.

Quality:..... The plant material supplied should be visibly healthy, not lacking in vigour or affected by any important pest or disease, especially virus.

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 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 reference 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.

It is the responsibility of Examination Office to keep the variety collection up to date.

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.

3. Characteristics to be used:

The characteristics to be used in DUS tests and preparation of descriptions shall be those referred to in Annex 1. 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 later 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 characters used for grouping are the following:

- (a) Flower: type (characteristic CPVO 11 – UPOV 15)
- (b) Petal: number of colours (characteristic CPVO 20 – UPOV 24)
- (c) One coloured varieties only: Petal: colour of upper side (characteristic CPVO 21 – UPOV 25) with the following groups:
 - Gr. 1: white
 - Gr. 2: yellow
 - Gr. 3: light green
 - Gr. 4: pink
 - Gr. 5: orange
 - Gr. 6: red
 - Gr. 7: purple
 - Gr. 8: violet

- (d) Bi-coloured varieties only: Petal: main colour of upper side (characteristic CPVO 22 – UPOV 26) with the following groups:
- Gr. 1: white
 - Gr. 2: yellow
 - Gr. 3: light green
 - Gr. 4: pink
 - Gr. 5: orange
 - Gr. 6: red
 - Gr. 7: purple
 - Gr. 8: violet
- (e) Bi-coloured varieties only: Petal: secondary colour of upper side (characteristic CPVO 23 – UPOV 27) with the following groups:
- Gr. 1: white
 - Gr. 2: yellow
 - Gr. 3: light green
 - Gr. 4: pink
 - Gr. 5: orange
 - Gr. 6: red
 - Gr. 7: purple
 - Gr. 8: violet
- (f) Petal: colour of base (characteristic CPVO 27 – UPOV 31)
- (g) Time of beginning of flowering (characteristic CPVO 32 – UPOV 36)

5. Trial designs and growing conditions

The minimum duration of tests will normally be one growing cycle if the results on distinctness and uniformity are conclusive. 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 40 plants for vegetatively propagated varieties and 60 plants for seed propagated varieties. Separate plots for observation and for measuring can only be used if they have been subject to similar environmental conditions.

All observations on single plants for vegetatively propagated varieties determined by measurement or counting should be made on 20 plants or 20 parts taken from each of 20 plants and any other observations made on all plants in the test during flowering time.

All observations on single plants for seed propagated varieties determined by measurement or counting should be made on 40 plants or 40 parts taken from each of 40 plants and any other observations made on all plants in the test during flowering time.

The test should normally be conducted at one place.

The test should be carried out in the glasshouse, under conditions ensuring normal growth.

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 of vegetatively propagated varieties and seed-propagated varieties which are self-pollinated, a population standard of 2% with an acceptance probability of at least 95% should be applied.

For a sample size between 19 and 41 plants for vegetatively propagated varieties, only 2 off-types are allowed.

For a sample size between 42 and 69 plants for seed propagated varieties which are self-pollinated, only 3 off-types are allowed.

For the assessment of uniformity of seed propagated open pollinated and hybrid varieties, relative uniformity standards should be applied.

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 growing cycle 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 one growing cycle but in some cases two or more 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 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 and final report shall be sent by the Examination Office to the CPVO.

ANNEXES TO FOLLOW

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ANNEX II

Technical questionnaire

ANNEX I

TABLE OF CHARACTERISTICS

CPVO N°	UPOV N°	Characteristics	Examples	Note	
1.	1.	Plant: height	short	White Coronet	3
			medium	Deep Purple, Momo Sen	5
			tall	Yuki no Mine	7
2.	3.	Stem: number of nodes	few	White Coronet	3
			medium	Momo Sen	5
			many	Purple Robin	7
3.	7.	Stem: position of branching	upper part only	Purple Moon	1
			upper and middle part only	Momo Sen	2
			whole stem		3
4. (+)	8. (+)	Leaf: attitude relative to stem	semi-erect	White Coronet	1
			horizontal	Momo Sen	2
			semi-drooping		3
5. (+)	9. (+)	Leaf: length	short	White Coronet	3
			medium	Momo Sen	5
			long		7

CPVO N°	UPOV N°	Characteristics	Examples	Note	
6. (+)	10. (+)	Leaf: width	narrow	White Coronet	3
			medium	Momo Sen	5
			broad		7
7. (+)	11. (+)	Leaf: shape	lanceolate		1
			ovate	Momo Sen	2
			broad ovate		3
8.	12.	Leaf: bloom	absent or very weakly expressed		1
			weakly expressed		2
			strongly expressed		3
9.	13.	Leaf: intensity of green colour of upper side (without bloom)	light		3
			medium	Momo Sen	5
			dark		7
10.	14.	Flower buds: number	few		3
			medium		5
			many	Blue Coronet	7
11.	15.	Flower: type	single		1
			double		2

CPVO N°	UPOV N°	Characteristics	Examples	Note	
12.	16.	<u>Varieties with double flowers only:</u> Flower: number of petals	few	3	
			medium	King of Blue Flash	5
			many	Deep Purple	7
13.	17.	Flower: diameter	small	3	
			medium	Momo Sen	5
			large	Deep Purple	7
14. (+)	18. (+)	Flower: shape	campanulate	Momo Sen	1
			narrow funnel-shaped	Purple Comet	2
			wide funnel-shaped	Fuku Shihai	3
			saucer-shaped	Deep Purple	4
15.	19.	Petal: length	short	3	
			medium	Momo Sen	5
			long	Yuki no Mine	7
16.	20.	Petal: width	narrow	Deep Purple	3
			medium	Yuki no Mine	5
			broad		7

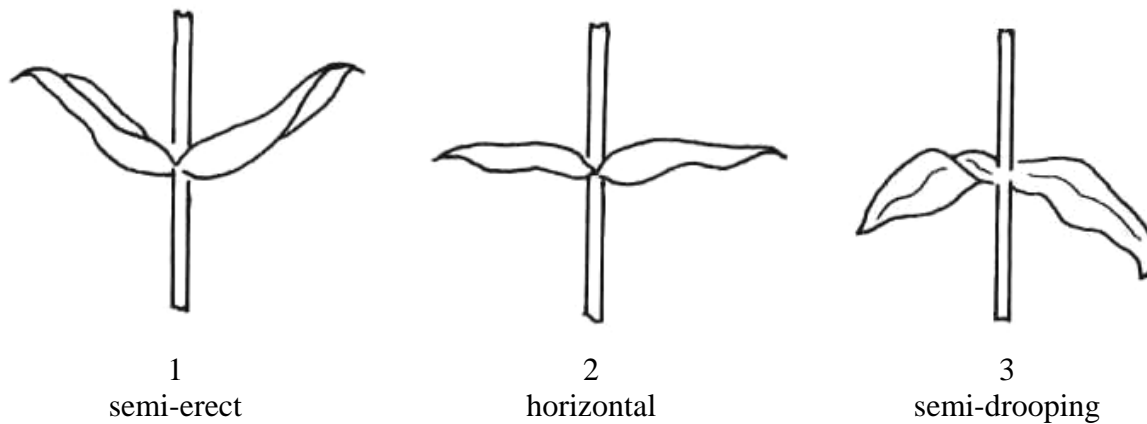
CPVO N°	UPOV N°	Characteristics	Examples	Note
17. (+)	21. (+)	Petal: shape of top margin	retuse	1
			flat	2
			rounded	3
			acute	4
18.	22.	Petal: undulation of margin	weak	Momo Sen 3
			medium	Yuki no Mine 5
			strong	Deep Purple 7
19.	23.	Petal: fringing of margin	absent or very weakly expressed	1
			weakly expressed	2
			strongly expressed	3
20.	24.	Petal: number of colours	one colored	1
			bi-colored	2
21.	25.	<u>One-colored varieties only:</u> Petal: colour of upper side	RHS Colour Chart (indicate reference number)	
22.	26.	<u>Bi-colored varieties only:</u> Petal: main colour of upper side	RHS Colour Chart (indicate reference number)	

CPVO N°	UPOV N°	Characteristics	Examples	Note	
23.	27.	<u>Bi-colored varieties only:</u> Petal: secondary colour of upper side			
			RHS Colour Chart (indicate reference number)		
24.	28.	<u>Bi-colored varieties only:</u> Petal: relative area of secondary colour			
			small	3	
			medium	5	
			large	7	
25. (+)	29. (+)	<u>Bi-colored varieties only:</u> Petal: colour pattern			
			spotted tip	1	
			picotee	Azuma no Yosooi	2
			shaded	Rainy Orange	3
			splashed	King of Blue Flash	4
			median stripe		5
26.	30.	Petal: main colour of lower side			
			RHS Colour Chart (indicate reference number)		
27.	31.	Petal: colour of base			
			green	Haku Sen	1
			violet	Fuku Shihai	2
			brown	Deep Purple	3
28. (+)	32. (+)	Calyx: length			
			short		3
			medium	Yuki no Mine	5
			long		7

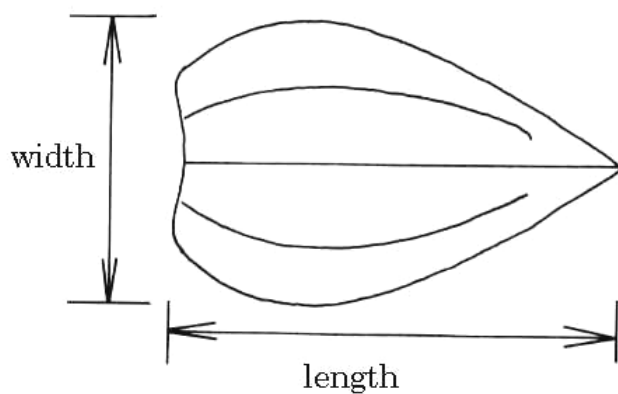
CPVO N°	UPOV N°	Characteristics	Examples	Note	
29.	33.	Calyx: anthocyanin coloration	absent	Haku Sen	1
			present	Shi Sen	9
30.	34.	Pedicel: length	short	White Coronet	3
			medium	Haku Sen	5
			long		7
31. (+)	35. (+)	Pistil: shape	type I		1
			type II		2
32.	36.	Time of beginning of flowering	early	Azuma no Yosooi	3
			medium	Haku Sen	5
			late	Fuku Shihai	7

EXPLANATIONS ON THE TABLE OF CHARACTERISTICS

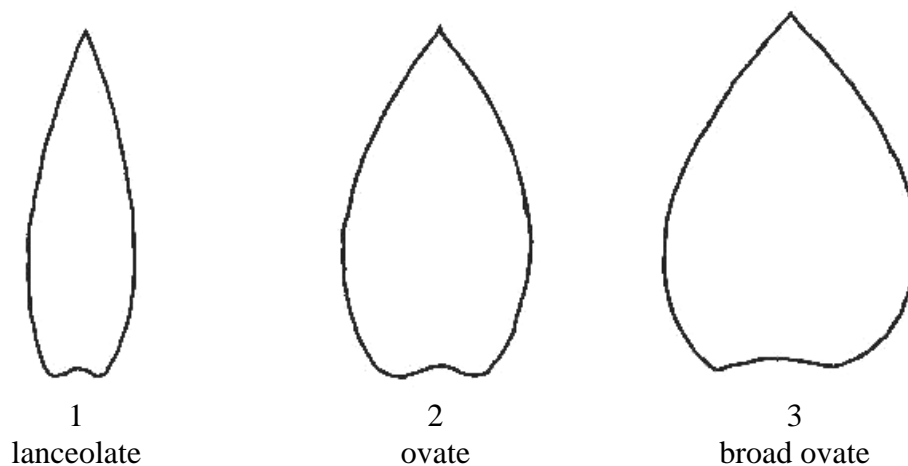
Ad. 4 (UPOV 8): Leaf: attitude relative to the stem



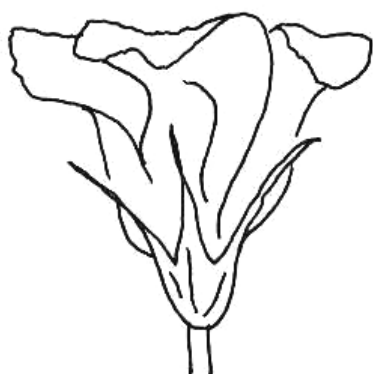
Ad.5 and 6 (UPOV 9 and 10): Leaf: length (5 - UPOV 9) and width (6 – UPOV 10)



Ad. 7 (UPOV 11): Leaf: shape



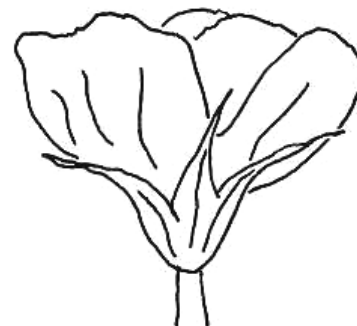
Ad. 14 (UPOV 18): Flower: shape



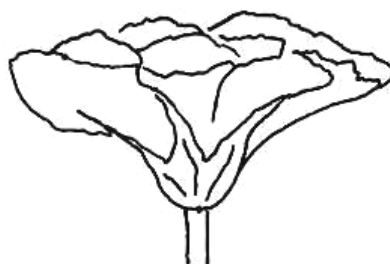
1
campanulate



2
narrow funnel-shaped

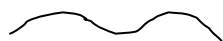


3
wide funnel-shaped

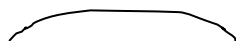


4
saucer-shaped

Ad. 17 (UPOV 21): Petal: shape of top margin



1
retuse



2
flat

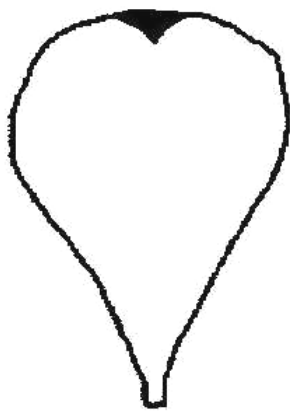


3
rounded

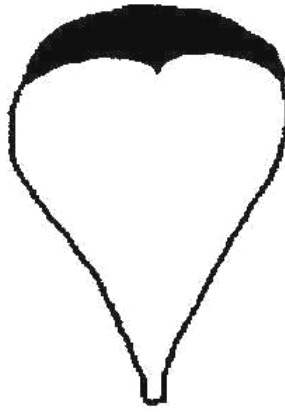


4
acute

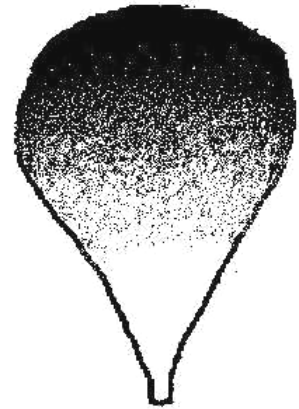
Ad. 25 (UPOV 29): Bi-colored varieties only: Petal: colour pattern



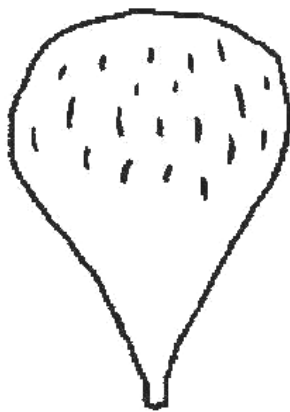
1
spotted tip



2
picotee



3
shaded

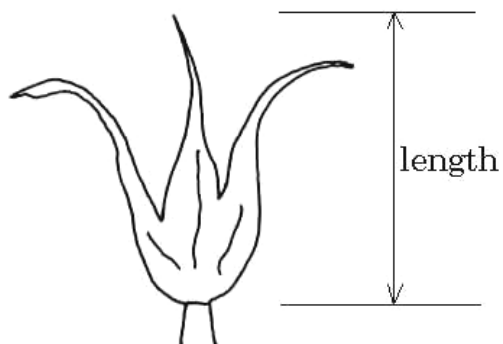


4
splashed

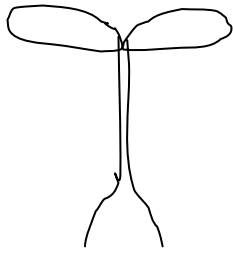


5
median stripe

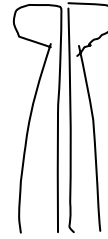
Ad. 28 (UPOV 32): Calyx: length



Ad. 31 (UPOV 35): Pistil: shape



1
type I



2
type II

LITERATURE

Kiyoshi Okawa, 1992: Eustoma (Torukogikyo) Seibunndo-Shinkosya Co., Tokyo, JP.

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

Eustoma grandiflorum (Raf.) Shinnery

EUSTOMA (LISIANTHUS)

Species (indicate)

- 2. 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. 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 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) []

4.2 Method of propagation

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

4.3 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)..... []

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

.....

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 (11) Flower: type	single	1 []
	double	2 []
5.2 (20) Petal: number of colours	one colored	1 []
	bi-colored	2 []
5.3i (21) <u>One-colored varieties only:</u> Petal: colour of upper side		
	RHS Colour Chart (indicate reference number)	
	

	Characteristics	Example varieties	Note
5.3ii (21)	<u>One-colored varieties only: Petal:</u> colour of upper side white yellow light green pink orange red purple violet Other colour (indicate)		1 [] 2 [] 3 [] 4 [] 5 [] 6 [] 7 [] 8 [] 9 []
5.4 i (22)	<u>Bi-colored varieties only: Petal: main</u> colour of upper side RHS Colour Chart (indicate reference number)		
5.4 ii (22)	<u>Bi-colored varieties only: Petal: main</u> colour of upper side white yellow light green pink orange red purple violet Other colour (indicate):		1 [] 2 [] 3 [] 4 [] 5 [] 6 [] 7 [] 8 [] 9 []

	Characteristics	Example varieties	Note
5.5i (23)	<u>Bi-colored varieties only:</u> Petal: secondary colour of upper side RHS Colour Chart (indicate reference number) 		
5.5 ii (23)	<u>Bi-colored varieties only:</u> Petal: secondary colour of upper side white yellow light green pink orange red purple violet Other colour (indicate):		1 [] 2 [] 3 [] 4 [] 5 [] 6 [] 7 [] 8 [] 9 []
5.6 (27)	Petal: colour of base green violet brown	Haku Sen Fuku Shihai Deep Purple	1 [] 2 [] 3 []
5.7 (36)	Time of beginning of flowering early medium late	Azuma no Yosooi Haku Sen Fuku Shihai	3 [] 5 [] 7 []

6. Similar varieties and differences from these varieties:

Denomination of similar variety	Characteristic in which the similar variety is different ¹⁾	State of expression of similar variety	State of expression of candidate variety
---------------------------------	--	--	--

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¹⁾ 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

A representative printed-out colour photo of the variety **must** be added to the Technical Questionnaire.

7.1 Resistance to pests and diseases

.....

7.2 Use of the variety

Pot plant []

Cut flower []

Other (specify) []

7.3 Special conditions for the examination of the variety

YES, please specify:

NO

7.4 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.

