Guidelines

For the Conduct of Test for
Distinctiveness, Uniformity & Stability
on

Knol khol
(*Brassica oleracea* var. *gongylodes* L.)

PPVFRA

Protection of Plant Varieties and Farmers' Rights Authority
(A Statutory Body created by an Act of Parliament)
Government of India, New Delhi

CONTENTS

I. Subject

These test guidelines shall apply to all varieties, parental lines and hybrids of Knol khol (*Brassica oleracea* var. *gongylodes* L.)

II. Planting Material Required

1. For all varieties, the planting materials are to be supplied in the form of seeds and should display all characteristics of the variety required for DUS testing. If, however, any material for DUS test has a specific requirement for the expression of characters, the same shall be specified by the applicant.

2. The Protection of Plant Varieties & Farmers' Rights Authority (PPVFRA) shall decide when, where and in what quantity and quality the seed material are required for testing of a variety denomination for registration under the Protection of Plant Varieties and Farmers' Rights (PPVFR) Act, 2001. Applicants submitting such seed material from a country other than India shall make sure that all customs and quarantine requirements stipulated under relevant national legislations and regulations are complied with.

3. The planting material (seed) supplied should be healthy, vigorous and free from pests and diseases. The seed should meet the minimum requirements for germination capacity (70%), moisture content (7%) and purity (98%) prescribed for certified seed in India.

4. The seed must not have been treated with any chemicals and bio-physical agents unless the competent authority allows or requests such treatment. If it has been treated, full details of the treatment must be provided along with the application.

5. The minimum quantity of seed for each variety to be submitted by the applicant shall be as per the following:

    - **New varieties:** 20 g divided equally in 10 packets
    - **Extant varieties**

    a) Varieties notified under Seeds Act, 1966: 4 g divided equally in 2 packets.

    b) Varieties about which there is a common knowledge: 10 g divided equally in 5 packets.

    c) Farmers' varieties: 10 g divided equally in 5 packets.

In case of hybrid, each of the parental lines is to be submitted as 4 g divided equally in 2 packets.

III. Conduct of tests

1. The minimum duration of the DUS test shall normally be as per following (sowing to harvest of seeds) with reference to the ecosystem of the candidate variety:
    - **New varieties:** Two seasons at two similar growing locations.
    - **Varieties of common knowledge/ farmers' varieties:** One season at two locations.
2. The test shall normally be conducted at least at two test locations. If any essential characteristic of the candidate variety is not expressed for observation/measurement at these locations, the variety should be considered for further examination at another appropriate test site or under special test protocol on the request expressed by the applicant for which additional quantity of seeds shall be required.
3. The field test shall be carried out under conditions favoring normal growth and expression of all test characteristics. The size of the plot shall be such that the plants or the parts of plants could be removed for measurement and observation without prejudicing the observations on the standing plants until the end of the growing period. Each test should include a minimum of 150 plants, in the plot size and planting space specified below across three replications. Separate plots for observation and for measurement can only be used if they have been subjected to similar environmental conditions. All the replications shall be sharing similar environmental conditions of the test locations.

    The test plot design shall be as follows-

Bed Size	:	3.0 m × 3.0 m
Number of rows	:	06
Row length	:	3.00 m
Row to row distance	:	50 cm
Plant to plant distance	:	30 cm
Number of replications	:	03
Design	:	RBD
Number of Locations	:	02

Number of plants per replication : 60

(Observations not to be recorded on plants in border rows)

4. Additional test protocols for special test shall be established by the PPV&FR Authority.

IV. Methods and observations

1. The characteristics described in the Table of characteristics (Section VII) shall be used for the DUS testing of varieties.

2. For the assessment of distinctiveness and stability, observations should be made on 30 randomly selected plants or parts of 30 plants, which shall be equally divided among 3 replications (10 plants per replication).

3. For the assessment of uniformity of characteristics on the plot as a whole (visual assessment by a single observation on a group of plants or parts of plants), a population standard of 1% with an acceptance probability of at least 95% should be applied. In the case of a sample size of 150 plants, the number of off-types should not exceed 2.

4. All observations on the plant, knob and the leaf should be made on plants fully developed in the vegetative stage. All observations on the leaf should be made on the leaves of middle whorls.

5. For the assessment of colour characteristics, the latest Royal Horticultural Society (RHS) colour chart shall be used.

V. Grouping characteristics

1. The candidate varieties for DUS testing should be divided into groups to facilitate the assessment of Distinctiveness. Characteristics which are known from experience not to vary, or to vary only slightly within a variety and which in their various states be fairly evenly distributed across all varieties in the collection are suitable for grouping purposes.
2. The following characteristics are proposed to be used for grouping Knol khol varieties:
    a) Plant: Attitude of leaves (Characteristic 3)
    b) Knob: Width (Characteristic 17)
    c) Knob: Shape in longitudinal section (Characteristic 18)
    d) Knob: Colour of skin (Characteristic 19)

VI. Characteristics and Symbols

1. To assess Distinctiveness, Uniformity and Stability, the characteristics and their states as given in the Table of characteristics should be used.
2. Notes (1-9) shall be used to describe the state of each character for the purpose of digital data processing and these notes shall be given opposite the states of the different characteristics.
3. **Legend**

   (*) Characteristics that should be observed during every growing season on all varieties and shall always be included in the description of the variety, except when the state of expression of any of these characters is rendered impossible by a preceding phenological characteristic or by environmental conditions of the testing region. Under such exceptional situation, adequate explanation should be provided.

   (+) See explanations on the Table of Characteristics in Section VIII. It is to be noted that for certain characteristics the plant parts on which observations to be taken are given in the explanation of figure(s) for clarity and not for the colour variation.

4. The optimum stage of plant growth for assessment of each characteristic is indicated in the sixth column of Table of Characteristics.

Description	**Code**
Seedling stage	10
Knob maturity stage	40
Flowering stage (50% plants with at least one open flower)	60

Characteristics containing the following key in the first column of the Table of characteristics shall be examined as indicated below:

QL: Qualitative characteristic

PQ: Pseudo-qualitative characteristic

QN: Quantitative characteristic

QL: Qualitative characteristics are those that are expressed in discontinuous states (e.g. Knob: Colour of skin, Leaf: Colour, etc.). These states are self-explanatory and independently meaningful. All states are necessary to describe the full range of the characteristic and every form of expression can be described by a single state. As a rule, the characteristics are not influenced by environment.

PQ: In Pseudo-qualitative characteristics, the range of expression is at least partly continuous, but varies in more than one dimension (e.g. Knob: Shape in longitudinal section: Transverse narrow Elliptic (3), Transverse broad Elliptic (5), Circular (7), Broad elliptic (9), and cannot be adequately described by just defining two ends of a linear range. In a similar way to qualitative (discontinuous) characteristics, each individual state of expression needs to be identified to adequately describe the range of the characteristic.

QN: Quantitative characteristics are those where the expression covers the full range of variation from one extreme to the other. The expression can be recorded on a one-dimensional, continuous or discrete, scale. The range of expression is divided into a number of states for the purpose of description (e.g. Leaf: Length of blade: short (3), medium (5), and long (7).

5. Type of assessment of characteristics indicated in column 7 of the Table of characteristics is as follows:

MG: Measurement by a single observation of a group of plants or parts of plants

MS: Measurement of a number of individual plant or part of plants

VG: Visual assessment by a single observation of a group of plants or parts of plants

VS: Visual assessment by observation of individual plants or parts of plants

VII. Table of Characteristics

S. No	**Characteristics**	**States**	**Note**	**Example varieties**	**Stage of observation**	**Type of assessment**
1. (+) (*) (QL)	Seedling: Anthocyanin colouration of hypocotyl	Absent	1	White Vienna, IC-0650630	10	VG
		Present	9	G-40, IC-0650632		
2. (+) (QN)	Plant: Height (cm)	Short (< 60 cm)	3	White Vienna, Pusa Virat	40	MS
		Medium (60 - 70 cm)	5	IC-0650630, IC-0650632		
		Tall (>70 cm)	7	IC-0650637, IC-0650658		
3. (*)	Plant: Attitude of leaves	Erect	3	IC-0650628, IC-0650637	40	VG

(+) (QL)		Semi Erect	5	White Vienna, Pusa Virat		
		Horizontal	7	Palam Tender Knob		
4. (*) (+) (QN)	Leaf: Length of blade (cm)	Short (< 20 cm)	3	White Vienna, Palam Tender Knob	40	MS
		Medium (20-30 cm)	5	Purple Vienna, Pusa Virat		
		Long (> 30 cm)	7	IC-0650627, IC-0650635		
5. (*) (+) (QN)	Leaf: Width of blade (cm)	Narrow (< 15 cm)	3	White Vienna, Pusa Virat	40	MS
		Medium (15-25 cm)	5	Purple Vienna, IC-0650627		
		Broad (> 25 cm)	7	IC-0650637, IC-0650643		
6. (+) (QL)	Leaf: Shape of blade	Elliptic	1	Purple Vienna, IC-0650644	40	VG
		Deltoid	3	IC-0650656, IC-0650657		
		Ovate	5	White Vienna, Pusa Virat		
		Broad ovate	7	IC-0650627, IC-0650637		
		Others	9	-		
7. (*) (+) (QL)	Leaf: Margin	Crenate	3	Pusa Virat, IC-0650627	40	VG
		Dentate	5	Purple Vienna, Palam Tender Knob		
		Lobed	7	White Vienna, G-40		
		Others	9	-		

8. (+) (QL)	Leaf: Shape of apex	Acute	3	G-40, IC-0650650	40	VG
		Obtuse	5	White Vienna, Purple Vienna		
		Others	7	-		
9. (+) (QL)	Leaf: Undulation of margin	Absent	1	Purple Vienna, IC-0650627	40	VG
		Present	9	White Vienna, Pusa Virat		
10. (QL)	Leaf: Waxiness	Absent	1	-	40	VG
		Present	9	White Vienna, Purple Vienna		
11. (+) (QL)	Leaf: Blistering	Absent	1	White Vienna, IC-0650656	40	VG
		Present	9	Purple Vienna, Pusa Virat		
12. (*) (QL)	Leaf: Colour	Yellow Green (144B)	3	IC-0650653	40	VG
		Green (143A/B/C, 142A/ 141C)	5	White Vienna, Purple Vienna		
		Purple (75A)	7	IC-0650629		
13. (*) (QL)	Leaf: Colour of midrib	Yellow Green (N144C)	3	IC-0650640	40	VG
		Green (141D, 142A/B/C/D)	5	White Vienna, Pusa Virat		
		Purple (N78A/B/C, 76D, 77B/C/D)	7	Purple Vienna, IC-0650628		
14.	Leaf: Length of petiole (cm)	Very short (<10 cm)	1	Pusa Virat, G-40	40	MS

(+) (QN)		Short (10-15 cm)	3	White Vienna, Palam Tender Knob		
		Medium (15-20 cm)	5	IC-0650629, IC-0650630		
		Long (20-25 cm)	7	IC-0650632, IC-0650642		
		Very long (> 25 cm)	9	IC-0650637, IC- 0650655		
15. (*) (QL)	Leaf: Colour of petiole	Yellow Green (144 A/D)	3	IC-0539756, IC-0650645	40	VG
		Green (142 A/B/C, 143 C/D)	5	White Vienna, Pusa Virat		
		Purple (N78 A/C, 79 A/C, N79 9A)	7	Purple Vienna, IC-0650628		
16. (*) (+) (QN)	Knob: Length (cm)	Short (< 5 cm)	3	IC-0650627, IC-0650632	40	MS
		Medium (5-7 cm)	5	White Vienna, Purple Vienna		
		Long (> 7 cm)	7	Pusa Virat, IC-0650636		
17. (*) (+) (QN)	Knob: Width (cm)	Small (< 5 cm)	3	IC-0650628, IC-0650637	40	MS
		Medium (5-8 cm)	5	Purple Vienna, Palam Tender Knob		
		Large (> 8 cm)	7	White Vienna, Pusa Virat		
18. (*) (+) (QL)	Knob: Shape in longitudinal section	Transverse Narrow Elliptic	3	White Vienna, Purple Vienna	40	VS
		Transverse Broad Elliptic	5	Pusa Virat, G-40		
		Circular	7	Palam Tender		

				Knob, IC-0650627		
		Broad elliptic	9	IC-0650628, IC-0650636		
19. (*) (QL)	Knob: Colour of skin	Yellow green (144B/C/D, N144C/D, 145A, 149D)	3	White Vienna, G-40	40	VG
		Green (142A/D, 143C/D)	5	Pusa Virat, IC-0650629		
		Purple (79A/B/C/D, N78C/D)	7	Purple Vienna, IC-0650628		
20. (*) (+) (QL)	Knob: Shape of apex	Indented	3	-	40	VG
		Level	5	Purple Vienna, IC-0650645		
		Raised	7	White Vienna, G-40		
21. (*) (+) (QL)	Knob: Density of crown (Number of leaves per cm^2)	Sparse (<3)	3	White Vienna, Purple Vienna	40	VG
		Medium (3-5)	5	Pusa Virat, G-40		
		Dense (>5)	7	IC-0650627, IC-0650628		
22. (+) (QN)	Harvest maturity	Early (<50 days)	3	Palam Tender Knob, IC-0650627	40	VG
		Medium (50-60 days)	5	White Vienna, Purple Vienna		
		Late (>60 days)	7	Pusa Virat, IC-0650643		
23. (+) (QN)	Flowering: Days to 50% flowering	Early (<115)	3	G-40, IC-0650630	60	VG
		Medium (115-130)	5	White Vienna, Palam Tender Knob		
		Late (>130)	7	Purple Vienna,		

				Pusa Virat		
24. (*) (QL)	Flower: Colour of petals	White	1	-	60	VG
		Vivid Greenish Yellow (2A)	2	Pusa Virat		
		Brilliant Greenish Yellow (3A, 3B, 4A, 5A, 5B)	3	White Vienna, Purple Vienna		
25. (*) (QL)	Flower: Male sterility	Absent	1	White Vienna, Palam Tender Knob	60	VG
		Present	9	-		

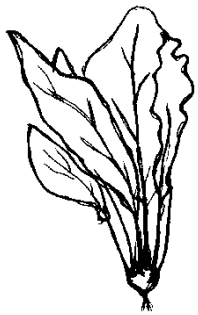
VIII. Explanation for the Table of Characteristics

Characteristic 1: Seedling: Anthocyanin colouration of hypocotyl
(To be observed after 3-4 days of germination)

Characteristic 2: Plant: Height

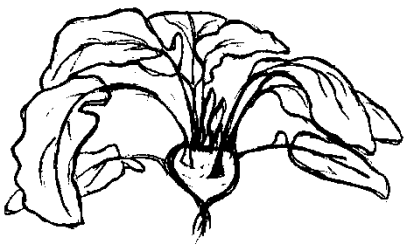
(Plant height is measured from soil surface to the tip of tallest leaf)

Characteristic 3: Plant: Attitude of leaves



Erect (3)

Semi spreading (5)

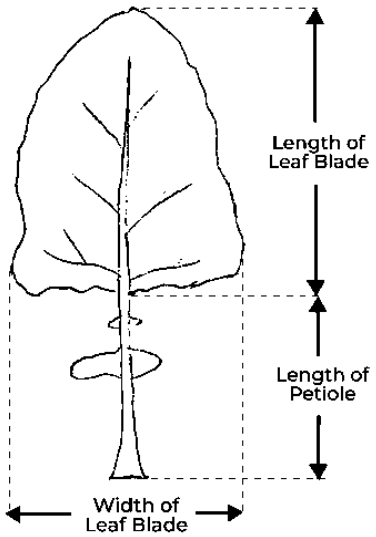


Spreading (7)

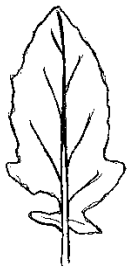
Characteristic 4: Leaf: Length of blade

Characteristic 5: Leaf: Width of blade (To be measured at the broadest part of leaf)

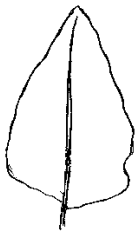
Characteristic 14: Leaf: Length of petiole



Characteristic 6: Leaf: Shape of leaf blade

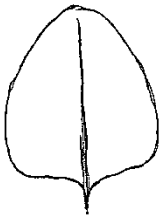


Elliptic (1)



Deltoid (3)

Ovate (5)



Broad ovate (7)

Characteristic 7: Leaf: Margin

Crenate (3)

Dentate (5)

Lobate (7)

Characteristic 8: Leaf: Shape of apex

Acute (3)

Obtuse (5)

Characteristic 9: Leaf: Undulation of margin

Absent (1)

Present (9)

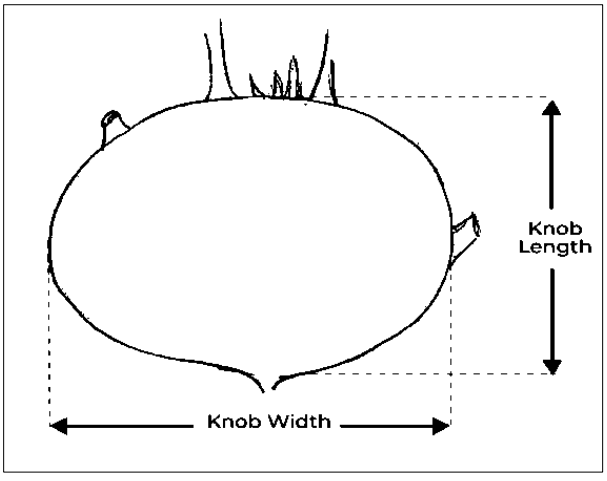
Characteristic 11: Leaf: Blistering

Absent (1)

Present (9)

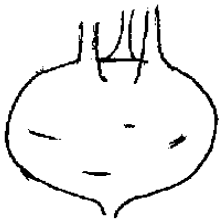
Characteristic 16: Knob: Length

Characteristic 17: Knob: Width (To be measured at the broadest part of knob)



Characteristic 18: Knob: Shape in longitudinal section

Transverse Narrow Elliptic (3)



Transverse Broad Elliptic (5)

Circular (7)

Broad elliptic (9)

Characteristic 20: Knob: Shape of apex

Indented (3)

Level (5)

Raised (7)

-

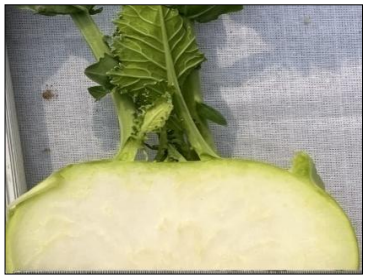
Characteristic 21: Knob: Density of crown

(Measured by counting number of leaves per cm^2 of crown)

Sparse (3)

Medium (5)

Dense (7)


Characteristic 22: Harvest maturity

(Duration from date of transplanting to the date of harvest)

Characteristic 23: Flowering: Days to 50% flowering

(Duration from date of replanting to the date when 50% plants in the population have at least one open flower)

IX Working Group details

The DUS test guidelines have been developed by the project team and refined by the Task Force constituted by the PPV & FR Authority for Knolkhol (*Brassica oleracea* var. *gongylodes* L.)

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X Name of DUS Test Centre

Nodal center

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