Guidelines
for the Conduct of Test for
Distinctiveness, Uniformity and Stability
on
Mesta (Kenaf & Roselle)

मेस्ता (केनाफ़ और रोज़ैल)

(*Hibiscus cannabinus* L. and *Hibiscus sabdariffa* L.)

[(हिबिस्कस कैनाबिनस (एल.) और हिबिस्कस सब्दारिफा (एल.)]

Protection of Plant Varieties and Farmers' Rights Authority (PPV & FRA)

(A Statutory Body created by an Act of Parliament)

Government of India, New Delhi

CONTENTS

DUS test guidelines for Mesta (*Hibiscus cannabinus* L. and *Hibiscus sabdariffa* L.)

I. Subject: These test guidelines shall apply to all varieties of Mesta viz., *Hibiscus cannabinus* L. and *Hibiscus sabdariffa* L., intra-specific hybrids, inter-specific hybrids, transgenic varieties and parental lines**.**

II. Seed material required:

1. The Protection of Plant Varieties and Farmers' Rights Authority (PPV & FRA) shall decide when, where and in what quantity and quality of the seed materials are required for testing a variety denomination applied for registration under the Protection of Plant Varieties and Farmers' Rights (PPV & FR) 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.

The minimum quantity of the seeds to be provided by the applicant is as follows:

i) New category: 600 g equally divided in 10 packets

ii) Extant category

   a) Varieties notified under *Seeds* Act, 1966: 120 g equally divided in 2 packets

   b) Farmers' varieties and varieties of common knowledge: 300 g equally divided in 5 packets

iii) For hybrid varieties, seeds of each of the parental lines are to be provided: 120 g divided in 2 packets

2. Seed should have at least 75% germination, 98% physical purity, highest genetic purity, uniformity, sanitary and phyto-sanitary standards. The moisture content of the seed shall not exceed 10 % to meet the safe storage requirement. The applicant shall also submit along with seed, a certified data on germination test made not more than one month prior to date of submission.

3. The seed material submitted shall not have been subjected to any chemical or bio-physical treatment

III. Conduct of tests

1. The minimum duration of DUS tests shall normally be at least two independent similar growing seasons for varieties under new category

2. and one season for farmers' varieties and varieties of common knowledge under extant category.

2. The tests shall normally be conducted at two test locations.

3. The field tests shall be carried out under conditions favouring normal growth and expression of all test characteristics. The size of the plots shall be such that plants or parts of plants could be removed for measurement and observation without prejudicing the other observations on the standing plants until the end of the growing period. Each test shall include a minimum of 600 plants in the plot size and planting space specified below across three replications.

4. **Test plot design**

i) Design : RCBD
ii) Number of rows : 5
iii) Row length : 5 m
iv) Row to row distance : 40 cm
v) Plant to plant distance : 10 cm
vi) Expected number of plants/replication : 250
vii) Number of replication : 3

5. Observations shall not be recorded on plants in border rows

6. Additional tests protocols for special purpose shall be established by the PPVFR Authority

IV. Methods and observations

- The characteristics described in the Table of characteristics (Section VII) shall be used for the testing of varieties for the standardization of DUS characters.
- Varieties of *H. cannabinus*L. shall be compared with varieties of *H. cannabinus*L. and varieties of *H. sabdariffa* L. shall be compared with varieties of *H. sabdariffa* L.
- For the assessment of distinctiveness and stability observations shall be made on at least 30 plants or parts of 30 plants, which shall be equally divided among three replications (10 plants per replication).
- For the assessment of uniformity of characteristics on the plot as a whole (visual assessment by a single observation of a group of plants or parts of

plants), the number of aberrant plants should not exceed 1% (maximum 11 off type plants allowed in a sample of 600 plants with an acceptance probability of 95%).

- For the assessment of all colour characteristics, the latest Royal Horticultural Society (RHS) colour chart shall be used
- All leaf characteristics shall be observed at full foliage stage i.e., around 60 days after sowing. Observations of five leaves starting from fully expanded sixth leaf from the top of the main branch (6^{th} leaf from top to 10^{th} leaf downward**)** shall be taken.
- All observations related to flower will be recorded after full opening of flower at 50% flowering stage.
- The optimum stage for the observation of each characteristic during the plant development/growth is indicated by a decimal code number in the 6^{th} column of Table of characteristics. The relevant growth stages corresponding to these decimal code numbers are described below:

Growth stage	**Code**
Seedling (up to two leaves stage)	5
Fully expanded foliage at 60 days after sowing	35
Appearance of flower bud (first flower bud just visible)	50
50% flowering (50% of the plants with at least one open flower)	60
Fruiting (capsules with full grown calyces)	90
Capsule harvest maturity	100

V. Grouping of varieties

1. The candidate varieties for DUS testing shall 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 are fairly evenly distributed across all varieties in the collection are suitable for grouping purposes.

- Varieties of *H. cannabinus* L. shall be compared with varieties of *H. cannabinus* L. and varieties of *H. sabdariffa* L. shall be compared with varieties of *H. sabdariffa* L.

Further for grouping of mesta varieties of a particular species, the following characteristics are proposed to be used:

i. Leaf: lobing pattern (Characteristic no. 2)
ii. Leaf: petiole pigmentation (Characteristic no.4)

iii. Stem: colour (Characteristic no. 8)

iv. Flower: petal colour (Characteristic no.15)

VI. Characteristics and symbols

1. To assess Distinctiveness, Uniformity and Stability, the characteristics and their states as given in the Table of Characteristics (Section VII) shall be used.

2. Notes (1 to 9) shall be used to describe the state of each character for the purposes of digital data processing and these notes shall be given against the states of each characteristic.

3. Legend:

(*) Characteristics that shall 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 the environmental conditions of the testing region. Under such exceptional situation, adequate explanation shall 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 observation to be taken are shown in the explanation or diagram(s) for clarity and not for colour variation.

4. Type of assessment of characteristics indicated in column 6 of 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 parts of plants

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

VS: Visual assessment by observations of individual plant or parts of plants

VII. Table of characteristics

Sl. No.	Characteristics	States	Notes	Example varieties		Stage of observation	Type of assessment
				H. cannabinus	*H. sabdariffa*		
1	2	3	4	5		6	7
1. (+)	Hypocotyl: Pigmentation	Absent	1	AMC 108, JRHC 3	HS 4288	5	VS
		Present	9	HC 583, MT 150	---		
*2. (+)	Leaf: lobing pattern	Unlobed	1	MT 150, JBMP 2	---	35	VS
		Lobed	5	JRHC 3, AMC 108,	Dark red roselle		
		Deeply lobed	9	---	AMV 5, CRIJAFR 8		
*3. (+)	Leaf: vein colour	Green (137A)	1	HC 583, JRM 3	Balijipeta local	35	VS
		Red (183A)	5	---	Dark red roselle		
		Variegated	7	AMC 108, JRHC 3	JRHS 1, AMV 7, JRR 17		
*4. (+)	Leaf: petiole Pigmentation	Absent	1	JRM 5, MT 150	Balijipeta local	35	VS
		Present	9	AMC 108, JRHC 3	HS 7910, HS 4288		
*5. (+)	Leaf: collar colour	Green (143C)	1	HC 583, MT 150	Balijipeta local	35	VS
		Red (183A)	5	AMC 108, JRHC 3	AMV 5, AMV 7,		
		Variegated	7	---	HS 4288, HS 7910		
6.	Leaf: Pubescence (adaxial)	Absent	1	HC 583, AMC 108	HS 7910, Dark red roselle	35	VS
		Present	9	---	AMV 5, AMV 7		

*7. (+)	Stipule: colour	Green (143C)	1	HC 583, MT 150	HS 4288, JRR 17	35	VS
		Greyish Reddish orange (174C)	5	AMC 108, JRHC 3	---		
		Variegated	7	---	AMV 7, HS 7910		
*8. (+)	Stem: Colour	Green (143C)	1	HC 583, MT 150	Balijipeta local	35	VG
		Coppery red (174A)	3	AMC 108, JRHC 3	---		
		Dark Red (187A)	5	---	Dark red roselle, AMV 7		
		Variegated	7	---	AMV 10, HS 7910		
		Others (green stem with red node)	9	---	HS 4288, JRR 17		
9.	Stem: pubescence	Absent	1	HC 583, AMC 108	Dark red roselle, HS 7910	50	VS
		Present	9	---	AMV 5, HS 4288		
*10. (+)	Stem: presence of Spine	Absent	1	CRIJAFK-1	Dark red roselle, HS 7910	50	VS
		Present	9	HC 583, MT 150	AMV 7, JRR 17		
11. **(+)**	Stem: branching habit	Non branching	1	HC 583, AMC 108	HS 7910, HS 4288	50	VG
		Branching	9	---	Dark red roselle		
*12. **(+)**	Plant height	Short (<2.5m)	3	---	Dark red roselle	50	MS
		Medium (2.5-3.5m)	5	HC 583, AMC 108 JRR 17	HS 4288, HS 7910		
		Tall (>3.5m)	7	JRM 5, JRHC 3	AMV 8, AMV 9		

*13. **(+)**	Fibre strength	Weak (<17 g/tex)	1	---	Dark red roselle	50	MG
		Average 17-20.9 g/tex)	3	---	HS 7910, HS 4288		
		Fairly good (21-24.9g/tex)	5	JRM 3, JRM 5	---		
		Good (25-28.9 g/tex)	7	JBM 81, HC 583	----		
		Very Good (29 g/tex and above)	9	---	---		
*14. **(+)**	Fibre fineness	Finer (<3.5 tex)	3	HC 583, AMC 108	HS 4288, Dark red roselle	50	MG
		Fine (3.5-4.2 tex)	5	MT 150	AMV 8, HS 7910		
		Coarse (>4.2 tex)	7	---	---		
*15. (+)	Flower: Petal colour	White	1	KIN 263	----	60	VS
		Pale yellow (4D)	3	-----	Dark red roselle		
		Creamy Yellow (2C, 2D)	5	HC 583, MT 150	HS 7910, AMV 7		
		Violet (94C)	7	JRHC 3	----		
*16. (+)	Flower: Eye zone colour	Yellow (2D)	3	KIJ 262	Balijipeta local	60	VS
		Purple (61A)	5	JBMP 2, HC 583	---		
		Red (N45)	7	---	HS 4288, HS 7910		
*17. (+)	Flower: stigma colour	Pale greenish yellow (2D)	3	---	Balijipeta local	60	VS
		Red (45B)	5	JRHC 3	---		
		Dark red (187B)	7	HC 583, JRM 3	AMV 7, HS 7910		

*18. (+)	Fruit: colour	Green (144D, 143C)	1	JRM 5, JBMP 2	Balijipeta local	90	VS
		Red (185B, 187C)	3	KEX 065	AMV 7, JRHS 1		
		Dark red (187B)	5	---	Dark red roselle		
		Variegated	7	---	HS 4288, JRR 17		
*19. (+)	Fruit: Pubescence	Smooth	1	---	Dark red roselle	90	VS
		Hairy	3	---	AMV 10, HS 7910		
		Bristled	5	HC 583, AMC 108	AMV 7, HS 4288		
20. **(+)**	Calyx length	Short (<30 mm)	3	JRM 3, JRHC 3	AMV 8, HS 4288	90	MS
		Medium (30-40 mm)	5	---	Dark red roselle		
		Long (>40 mm)	7	---	HSLC 1		
*21. (+)	Seed: Seed coat colour	Brownish grey (N200B)	3	JRM 5, JBMP 2	-	100	VG
		Greyish brown (166A)	5	-	RIJ 030		
		Brown (200C, 200D)	7	KIJ 072	AMV 8, HS 4288,		
*22.	Seed: Size (Thousand seed weight at 10% moisture content)	Small (≤20.0g)	3	---	HS 4288, AMV 3	100	MG
		Medium (20.1g-30.0g)	5	JBMP 2, MT 150	HS 7910, JRR 17		
		Large (>30.0g)	7	HC 583, JRHC 3	Dark red roselle		

VIII. Explanations on the Table of characteristics

Kenaf plant (flowering stage)

Roselle plant (pre-flowering stage)

Roselle plant (branching type)

Roselle plant (flowering stage)

Characteristic 1: Hypocotyl: Pigmentation

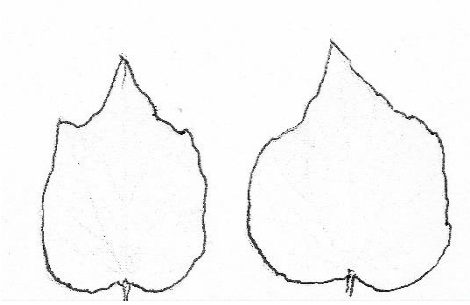
H. cannabinus *H. sabdariffa*



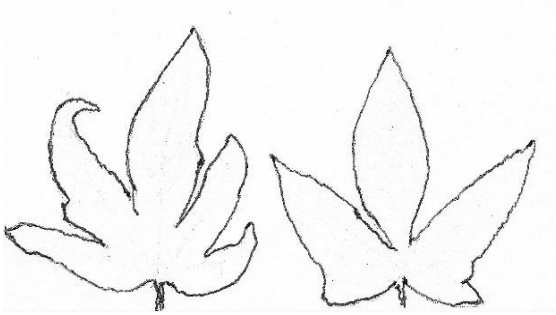
Absent (1) Present (9) Absent (1)

Characteristic 2: Leaf: lobing pattern

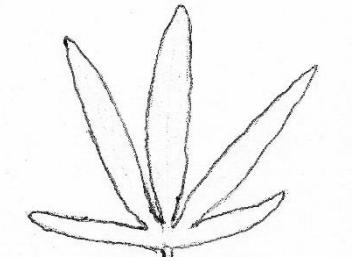
This characteristic shall be recorded on leaves (starting from 6thleaf from top of the main stem) at fully expanded foliage at 60 days after sowing.



Unlobed(1)



Lobed(5)



Deeply lobed(9)

Characteristic 3: Leaf: vein colour

H. cannabinus *H. sabdariffa*


Green (1)


Variegated (7)


Green (1)

Red (5)

Characteristic 4: Leaf: petiole Pigmentation

H. cannabinus *H. sabdariffa*


Absent (1)


Present (9)


Absent (1)


Present (9)

Characteristic 5: Leaf: collar colour

H. cannabinus *H. sabdariffa*


Green (1)


Red (5)


Green (1)


Red (5)


Variegated (7)

Characteristic 7: Stipule: colour

H. cannabinus *H. sabdariffa*


Green (1)


Greyish Reddish orange(5)


Green (1)


Variegated (7)

Characteristic 8: Stem: Colour

H. cannabinus *H. sabdariffa*


Green (1)


Coppery red (3)


Green (1)


Dark Red (5)


Variegated (7)


Others (green stem with red node) (9)

Characteristic 10: Stem: presence of Spine

H. cannabinus *H. sabdariffa*


Absent (1)

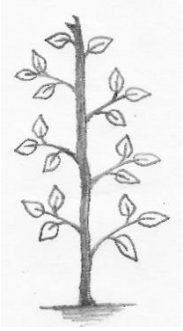

Present (9)


Absent (1)


Present (9)

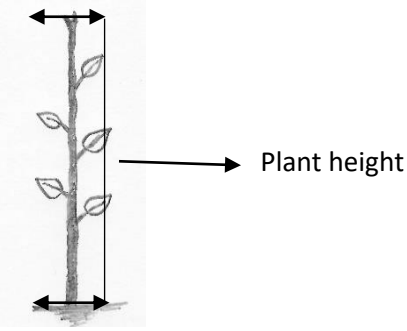
Characteristic 11: Stem: branching habit


Non branching(1)


Branching (9)

Characteristic 12. Plant Height

This characteristic shall be recorded as height of the main stem measured from ground level to the tip of plant (main stem) at 120 days (after appearance of first flower bud).



Characteristic 13. Fibre: strength (g/tex)

Sampling

Fibre strength can be determined by fibre bundle strength tester. For each variety 16 samples are prepared (fibre obtained from 10 well retted plants/ replication) for measuring fibre strength as followed in the case of AINP JAF trials.

Instrument: Automatic Electronic Fibre Bundle Strength Tester (Model NIRJAFT-AEFBST-MF01) [Developed by National Institute of Research for Jute and Allied Fibres Technology (NIRJAFT), Kolkata].

Methodology:

Fibre strength can be measured with the help of Fibre Bundle Strength Tester. Exactly 12.5 cm of fibre is cut from the middle portion of the reed. Weight of the cut portion of the fibre is taken. It should be within the range of 200 to 400 mg. Two clamps are placed on two slots fixed on the base of the instrument. They will be exactly 5 cm apart. Weighed fibre sample is put into the clump. The plungers of the two clamps automatically tightened in such a way that all the fibres should be parallel to each other with equal tension. The automatically operated fibre grips will now be closed and the sample is now under tension resulting from rotation of driving motors. If the sample breaks properly Sample Broken message will be displayed and test result will be displayed showing peak breaking load (Kg), elongation (%) and tenacity (g/tex).

Strength (g/tex) =125 × (Breaking Load in kg/ Wt. of the bundle in mg)

Characteristic 14. Fibre: Fineness (tex)

Fibre fineness may be measured from the replicated samples by Airflow method which is broadly followed everywhere for assessing fibre fineness in natural fibres. The principle of this method depends on the well knownKozeny's equation of airflow through a fibre plug.

Instrument: Digital Fineness Meter (Model NIRJAFT-DFM-MF01) [Developed by National Institute of Research for Jute and Allied Fibres Technology (NIRJAFT), Kolkata]

Methodology:

1. Sampling: Ten to fifteen portions of 10 cm length of jute fibre are taken from the middle portions of a reed (fibre obtained from 10 well retted plants/ replication) so that the approximate weight of the bundle is 8 g. The bundle of fibre is cleaned so as to free it from barky, specky and knotty spots. The bundle is then placed on a cutter and it is cut exactly 5 cm in length with the help of a chisel and hammer. The bundle is then weighed exactly to 3 g.

2. Bundle insertion into the cell: The weighed bundle is wrapped tightly on a piece of paper and introduced into the cylindrical fibre holder. The paper is then taken out and the fibre holder is fitted airtight in the fibre holder chamber.

3. Pressure adjustment and observation: Actual pressure is adjusted to 0.72 KPa with pressure adjustment knob. Then TEST switch is pressed to test the sample for its fineness, which will be displayed directly in tex value.

Characteristic 15: Flower: Petal colour

H. cannabinus *H. sabdariffa*


White (1)


Creamy yellow (5)


Violet (7)


Pale yellow (3)


Creamy yellow (5)

Characteristic 16: Flower: Eye zone colour

H. cannabinus *H. sabdariffa*


Yellow (3)


Purple (5)


Yellow (3)


Red (7)

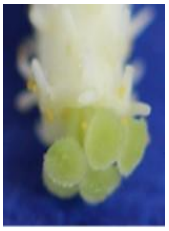
Characteristic 17: Flower: stigma colour

H. cannabinus

Red (5)

Dark red (7)

H. sabdariffa



Pale greenish yellow (3)

Dark red (7)

Characteristic 18: Fruit: colour

H. cannabinus

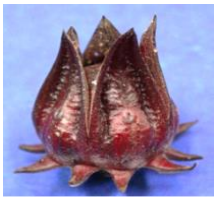
Green (1)

Red (3)

H. sabdariffa

Green (1)

Red (3)



Dark red (5)

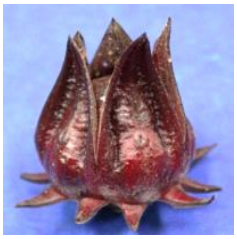
Variegated (7)

Characteristic 19: Fruit: Pubescence

H. cannabinus

Bristled (5)

H. sabdariffa



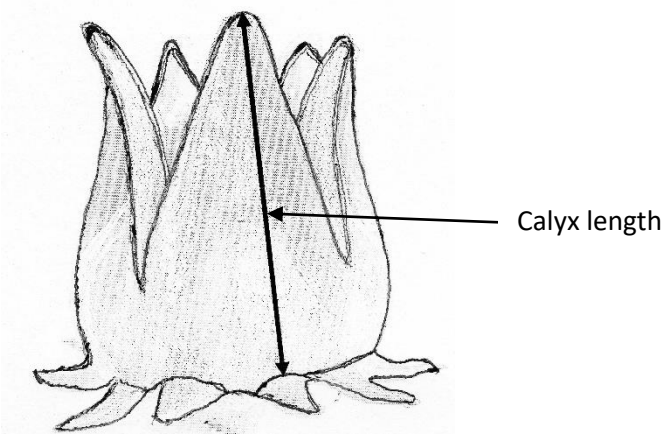
Smooth (1)

Hairy (3)

Bristled (5)

Characteristic 20. Calyx length

This characteristic should be recorded in full grown capsules. Calyces should be cut opened and from point of attachment at the base of capsule. Length should be measured from the base to the tip at middle portion of calyx lobe.



Calyx

Characteristic 21: Seed: Seed coat colour

H. cannabinus

Brownish grey (3)

Brown (7)

H. sabdariffa

Greyish brown (5)

Brown (7)

IX. DUS centres

Nodal centre	**Co-nodal Centre**
ICAR-CRIJAF, Saheb Bagan, Nilganj, Barrackpore, Kolkata 700121	Agricultural Research Station, ANGRAU, Amadalavalasa, A.P-532185

X. Working Group:

The test guidelines were developed by the ICAR-Central Research Institute for Jute and Allied fibres, Barrackpore, WB in association with Agricultural Research Station, ANGRAU, Amadalavalasa, A.P and the task force constituted by PPVFR Authority.

Following scientists were involved in the development of guidelines.

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3. Dr. L. Suryanarayana, Principal Scientist & Co-PI, ARS, Amadalavalasa

4. Dr. G. Sreenivas, Scientist& Co-PI, ARS, Amadalavalasa

Nodal officer/Principal Investigator

Dr. Amit Bera, Principal Scientist, ICAR- CRIJAF, Barrackpore

Co-Nodal officer/ Co-Principal Investigator

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Prof.Dilip Kumar De Former Associate Dean, College of Agriculture, (BCKV)	**Member**
Dr. N. Hari Satyanarayana Principal Scientist, RARS-ANGRAU, Lam, Guntur, AP	**Member**
Dr. C. S. Kar Principal Scientist, ICAR-CRIJAF, Barrackpore	**Member** (Director's nominee)
Dr. Amit Bera Principal Scientist , ICAR-CRIJAF, Barrackpore,	**Member**
Sh. D. R. Choudhury Joint Registrar, PPVFR Authority, New Delhi	**Member Secretary**