Draft

Guidelines
for the Conduct of Test for
Distinctiveness, Uniformity and Stability

On

Tetraploid Cotton

(*Gossypium hirsutum* L. and *G.barbadense* L.)

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

CONENTS Page

Tetraploid cotton

(*Gossypium hirsutum* L. and *G.barbadense* L.)

I. Subject

These test guidelines shall apply to all varieties of tetraploid cotton *viz*., *Gossypium hirsutum* L. and *Gossypium barbadense* L. intra-specific hybrids, inter-specific hybrids 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 material 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 seed to be provided by the applicant shall be 2000 gram in the case of the candidate variety or hybrid and 1000 gram for each of the parental line of the hybrid. Each of these seed lots shall be packed, sealed and properly labeled with details, in ten equal weighing packets and submitted in one lot. Parental line should be packed in one packed.
2. The seeds submitted shall have at least 75 % germination, 98 % physical purity, the 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. A certificate indicating germination percentage recorded not more than one month before the submission of sample shall be attached.
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.
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 visual observation at these locations, the variety shall be considered for further examination at another appropriate test site or under special test protocol on expressed request of the applicant.
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

the plant parts or 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 shall include a minimum of 300 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 replication shall be sharing similar environmental conditions of the test location.

4. Test plot design:

Number of rows	:	12
Row length	:	6 m
Row to row distance	:	90 cm
Plant to plant distance	:	60 cm
Expected plants/ replication	:	120
Number of plants / hill	:	1
Number of replications	:	3

5. Observations shall not be recorded on plants in border rows.
6. Additional test protocols for special purpose shall be established by the PPV & FR Authority.

IV. Methods and observations

1. The characteristics described in the Table of characteristics (see Section VII) shall be used for the testing of varieties, parental lines and hybrids for their DUS test.
2. For the assessment of Distinctiveness and Stability, observations shall be made on 30 plants or parts of 30 plants, which shall be equally divided among three 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 of a group of plants or parts of plants), a population standard of 0.5 % with an acceptance probability of at least 95 % shall be obtained. In the case of a sample size of 300 plants, the number of off types should not exceed 6.
4. All leaf characteristics shall be observed on the fourth fully expanded leaf from the top of the main stem at 50 % flowering stage.
5. For the assessment of colour characteristics, the latest Royal Horticultural Society (RHS) colour chart shall be used.
6. All observations on the flower shall be made on the first day of flowering and at anthesis.
7. Observations on the boll shall be made at full maturity and before boll bursting.

V. Grouping of varieties

1. The candidate varieties / hybrids for DUS testing belonging to *G. hirsutum*, *G. barbadense* and inter-specific (*G. hirsutum x G. barbadense*) hybrid will be tested separately.
2. 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 purpose.
3. The following characteristics are proposed to be used for grouping cotton varieties:

    i) Species
    ii) Leaf : Shape (Characteristic 8)
    iii) Flower: Petal colour (Characteristic 15)
    iv) Flower :Pollen colour (Characteristic 19)
    v) Boll : Shape (longitudinal section) (Characteristic 23)
    vi) Fibre : Length (Characteristic 33)

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 purpose 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.

4. A decimal code number in the sixth column of Table of Characteristics indicates the optimum stage for the observation of each characteristic during the growth and development of plant. The relevant growth stages corresponding to these decimal code numbers are described below:

Decimal Code for the growth stages:

Growth stage	Code
Seedling	5
Square formation	30
50% Flowering (at least one flower should have opened in 50% of the population in the plot)	40
Boll bursting	65
First picking (20% of bolls in each plant must have opened)	75
Final harvest	95

5. Type of assessment of characteristics indicated in column 7 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 plants 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 plants or parts of plants.

VII. Table of Characteristics

Sl. No.	Characteristics	States	Notes	Example varieties	Stage of obser vation	Type of assess ment
1	2	3	4	5	6	7
1	Hypocotyl: Pigmentation	Absent	1	DHY 286-1 (H)	5	VS
		Present	9	Sumangala (H)		
2 (*)	Leaf: Colour	Light green	1	Abadhita (H)	40	VS
		Green	2	Sumangala (H)		
		Light red	3			
		Dark red	4	BN Red (H)		
3 (*)	Leaf: Hairiness	Sparse	1	Suvin (BA)	40	VS
		Medium	5	LRA 5166 (H)		
		Dense	9	DHY 286-1 (H)		
4	Leaf: Appearance	Cup	1	Abhadita (H)	40	VS
		Flat	2	LRA 5166 (H)		
5	Leaf: Gossypol glands	Absent	1	Acala Glandless(H)	40	VG
		Present	9	LRA 5166 (H)		
6 (*)	Leaf: Nectaries	Absent	1	American Nectariless (H)	40	VG
		Present	9	Sumangala (H)		
7	Leaf: Petiole pigmentation	Absent	1	G. Cot. 12 (H)	40	VS
		Present	9	Surabhi (H)		
8 (*) (+)	Leaf: Shape	Palmate (Normal)	1	LRA 5166 (H)	40	VS
		Semi-digitate (Semi- Okra)	2	LHH 144(H)		
		Digitate (Okra)	3	PIL 43 (H)		
		Lanceolate (Super Okra)	4	Arizona Super Okra (H)		
9 (*)	Plant: Stem hairiness	Smooth	1	Suvin (BA)	30	VS
		Sparse	3	Narasimha (H)		
		Medium	5	MCU 5 (H)		
		Dense	7	DHY 286 (H)		
10	Plant: Stem	Absent	1	Surat Dwarf (H)	45	VS

	pigmentation	Present	9	BN Red (H)		
11	Plant: Height (cm)	Dwarf (< 60)	1		75	MS
		Semi dwarf (60 – 90)	3	70 E (H)		
		Medium tall (91 – 120)	5	Anjali (H)		
		Tall (121–150)	7	LRA 5166 (H)		
		Very tall (> 150)	9	Sumangala (H)		
12 (+)	Plant: Growth habit	Zero branching	1	P 4 (BA)	75	VG
		Compact (Spreading<30 cm)	3			
		Semi-spreading (31–60 cm)	5			
		Spreading(>60cm)	7			
13 (+) (*)	Bract: Type	Normal	3	LRA 5166 (H)	40	VS
		Frego	5	BN Frego (H)		
14	Flower: Time of flowering (50% of plants with at least one open flower)	Early (<50 days)	3	BN 1 (H)	40	VG
		Medium (50–60 days)	5	AC 738 (H)		
		Late (>60 days)	7	G 67 (H)		
15 (*)	Flower: Petal colour	Cream	1	LRA 5166 (H)	40	VS
		Yellow	2	Laxmi (H)		
		Deep Yellow	3	Suvin (BA)		
		Purple	4	BN Red (H)		
16 (*)	Flower: Petal spot	Absent	1	LRA 5166 (H)	40	VS
		Present	9	Suvin (BA)		
17 (*) (+)	Flower: Stigma	Embedded	3	Supriya (H)	40	VG
		Exerted	5	Surabhi (H)		
18	Flower: Anther filament colouration	Absent	1	Sumangala (H)	40	VG
		Present	9	G. Cot. 12 (H)		
19 (*)	Flower: Pollen colour	White	1	LH 900 (H)	40	VS
		Cream	2	Anjali (H)		
		Yellow	3	LRA 5166 (H)		

		Deep Yellow	4	Suvin (BA)		
		Purple	5	BN Red (H)		
20	Flower: Male sterility (Only for A and B lines)	Absent	1	MCU 5 VT (H)	40	VS
		Present	9	AK 32 A (H)		
21	Boll: Bearing habit	Solitary	1	Sumangala (H)	65	VS
		Cluster	9	P 4 (BA)		
22	Boll: Colour	Green	3	Sumangala (H)	65	VS
		Red	5	BN Red (H)		
23 (*) (+)	Boll: Shape (longitudinal section)	Round	3	Supriya (H)	65	VG
		Ovate	5	Surabhi (H)		
		Elliptic	7	Suvin (BA)		
24 (*)	Boll: Surface	Smooth	1	LRA 5166 (H)	65	VG
		Pitted	9	Suvin (BA)		
25 (*)	Boll: Prominence of tip	Blunt	1	Supriya (H)	65	VG
		Pointed	9	Surabhi (H)		
26 (*) (+)	Boll: Opening	Semi-open	3	Suvin (BA)	75	VG
		Open	5	LRA 5166 (H)		
27 (*)	Boll: Weight of seed cotton/boll (g)	Very small (<3.0)	1	Suvin (BA)	75	MS
		Small (3.0-4.0)	3	Bikaneri Narma(H)		
		Medium (4.1-5.0)	5	MCU 5 (H)		
		Large (5.1-6.0)	7	Supriya (H)		
		Very large (>6.0)	9	JK 572 (H)		
28 (*)	Seed: Fuzz	Naked	1	Suvin (BA)	95	VG
		Sparse	3			
		Medium	5	RHCb 001 (BA)		
		Dense	7	Sumangala (H)		
29 (*)	Seed: Fuzz colour	White	1	LRA 5166 (H)	95	VS
		Grey	2	RS 2013 (H)		
		Green	3	DCH 32 (HxBA)		
		Brown	4	Khandwa Brown (H)		
30 (*)	Seed: Index (100 seed wt in gram)	Very small (<5.0)	1		95	MS
		Small (5.0-7.0)	3			

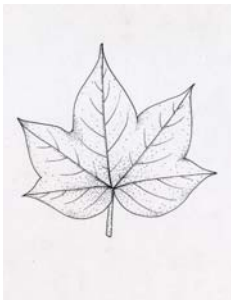
		Medium (7.1-9.0)	5	LRA 5166 (H)		
		Bold (9.1-11.0)	7	G 67 (H)		
		Very bold (>11.0)	9	VICH 5 (H x H)		
31 (*)	Ginning %	Very low(≤30)	1	Suvin (BA)	95	MG
		Low (31-32)	3	TCHB 213 (H x BA)		
		Medium (33-34)	5	MCU 5 VT (H)		
		High (35-36)	7	LRA 5166 (H)		
		Very high (≥37)	9	Supriya (H)		
32 (*)	Fibre: Colour	White	1	LRA 5166 (H)	75	VS
		Cream	2	B 82-1-1 (BA)		
		Green	3	Arkansas Green Lint (H)		
		Brown	4	Nankeen Spot(H)		
33 (*) (+)	Fibre: Length (2.5 % span length) (mm)	Short (≤20)	1	Sharada (H)	95	MG
		Medium (20.5- 24.5)	3	LRA 5166 (H)		
		Medium long (25.0 - 27.0)	5	Anjali (H)		
		Long (27.5 - 32.0)	7	Supriya (H)		
		Extra long (≥32.5)	9	MCU5 VT (H)		
34 (*) (+)	Fibre: Strength (g/tex)	Very Weak (≤16)	1		95	MG
		Weak (17.0- 20)	3	Sumangala (H)		
		Medium (21.0-24.0)	5	MCU 5 VT (H)		
		Strong (25.0 - 28.0)	7			
		Very Strong (≥29)	9	Suvin (BA)		
35 (+)	Fibre: Fineness (Micronaire value)	Very coarse (≥6.0)	1		95	MG
		Coarse (5.0-5.9)	3	RHC 004 (H)		
		Medium (4.0- 4.9)	5	Savita (H x H)		
		Fine (3.0-3.9)	7	MCU 5 VT (H)		

		Very fine (≤3.0)	9	Kasinath (HxB)		
36 (+)	Fibre: Uniformity (%)	Poor (<42)	1	AK 23 A (H)	95	MG
		Fair (42-43)	3	AK 32 B (H)		
		Average (44-45)	5	MCU 5 VT (H)		
		Good (46-47)	7	MCU 3 (H)		
		Excellent (>47)	9	70 E (H)		
37 (+)	Fibre: Maturity (%)	Very Immature (≤31)	1		95	MG
		Immature (32-49)	3			
		Average (50-65)	5	MCU 5 VT (H)		
		Good (66-80)	7	LRA 5166 (H)		
		Very Good (≥ 81)	9	RHC 004 (H)		

Note: (H) – *Gossypium hirsutum*, (BA) – *Gossypium barbadense*

VIII. Explanations and methods

Characteristic 8. Leaf: shape

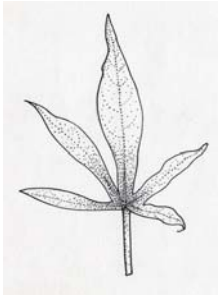


1

Palmate (Normal)

2

Semi digitate (Semi Okra)



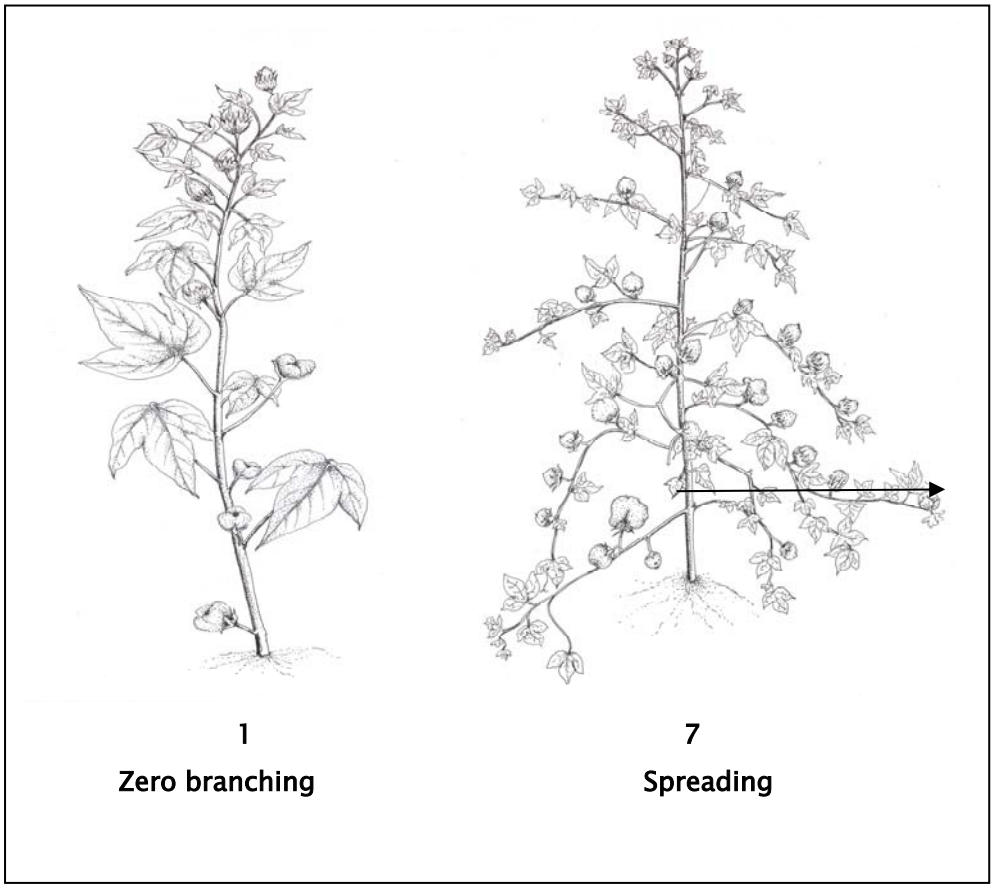
3

Digitate (Okra)

4

Lanceolate (Super Okra)

Characteristic 12. Plant: growth habit

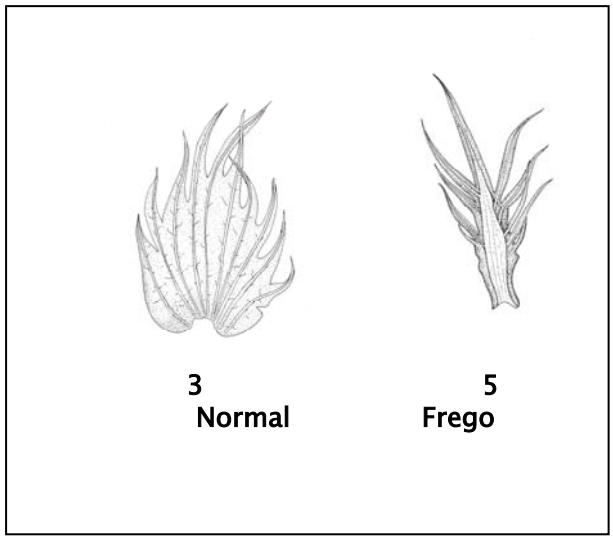


1
Zero branching

7
Spreading

Note: Length of longest sympodia will be measured.

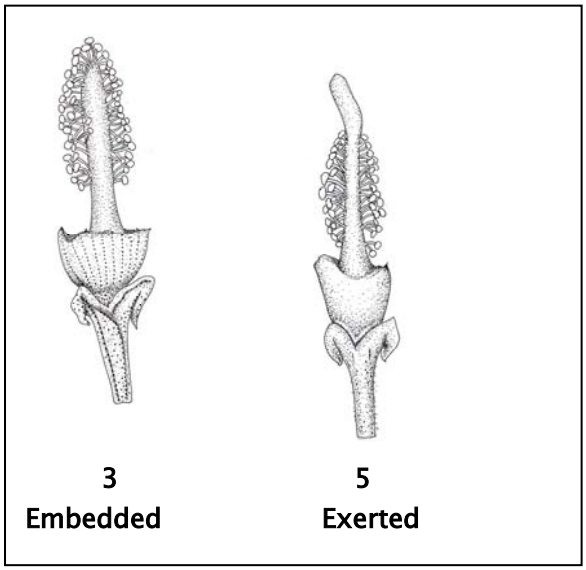
Characteristic 13. Bract: type



3
Normal

5
Frego

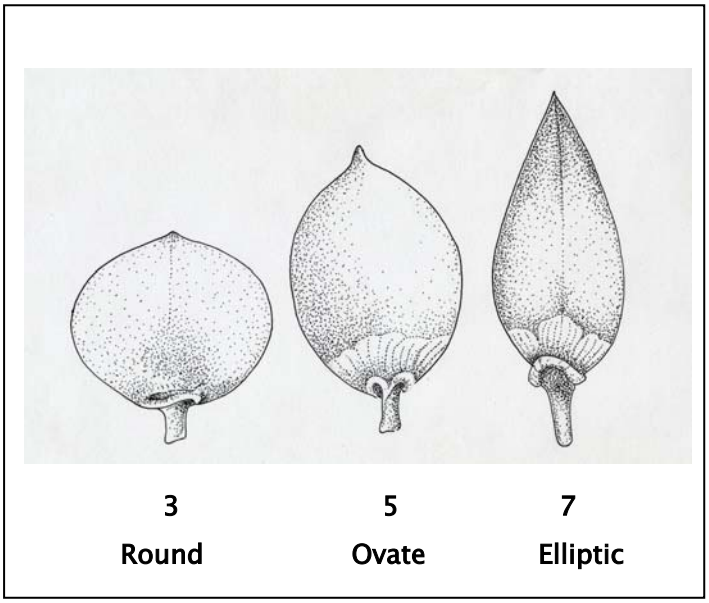
Characteristic 17. Flower: Stigma



3
Embedded

5
Exerted

Characteristic 23. Boll: Shape

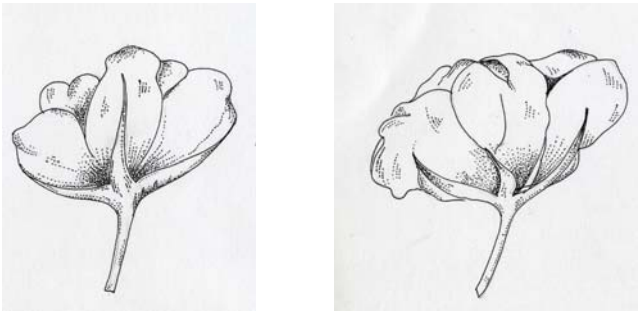


3
Round

5
Ovate

7
Elliptic

Characteristic 26. Boll: Opening



Semi open
3

Open
5

Characteristics 33. (2.5% Span length), 34. (Fibre strength), 35. (Fibre fineness), 36. (Fibre uniformity) and 37. (Fibre maturity)

The major fibre properties *Viz.,* 2.5% Span length, Fibre strength, Fibre fineness (Micronaire), Fibre uniformity and Fibre maturity (%) shall be determined under ICC mode using a standard High Volume Instrument (HVI).

Fifty gram of lint pooled equally from all the replications shall be used for determining the above fibre parameters. The samples should be conditioned for at least 2 hours at a room temperature of 27 $\pm$ 2°C and relative humidity of 65 $\pm$ 2 %. The moisture content of the sample should be around 7-8 %.

Calibration with HVI Calibration cotton supplied by the CIRCOT shall be done before testing the samples.

A fibro comb containing the test sample prepared with the help of a fibre sampler is placed in the comb track. The test cycle consists of automatic brushing of the sample in the fibro comb and placing it on the comb holder. The test specimen then moves through a light beam. Using the optical mass generated and the software installed, **2.5% Span length** and **Uniformity ratio** are derived.

After the test specimen passing through the sensor, the beard is positioned at the break point over a set of clamping jaws with 3 mm spacing between the jaws. The force required to break the beard is calculated in units of g/tex with the help of software and is recorded as **Fibre strength**.The cycle is repeated four times and the average values are taken.

To measure the **Fibre Fineness (Micronaire),** the lint sample is opened thoroughly after removing all the trash. Approximately 10 g of lint is weighed and inserted into the porosity chamber and the lid is closed. Compressed air is allowed to flow through the sample inside the chamber. From the measured values of mass and pressure, the microprocessor calculates the specific surface, which is converted into the Micronaire value. Using the Micronaire value the software also derives **Fibre maturity (%).**