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

on

Sugarcane
(Saccharum L.)

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

I. Subject

These test guidelines shall apply to all vegetatively propagated varieties, transgenics, hybrids and parental lines of Sugarcane (*Saccharum* L.).

II. Planting 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 planting 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 planting 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 planting material to be supplied by the applicant shall be 400 single bud setts in one submission only.

2. The planting material (seed cane) shall be supplied from pure 8-10 month old plants, preferably from the top cane portion, visibly healthy, not lacking in vigour, or affected by any pest or disease. It should not be obtained from *in vitro* propagation and shall possess highest genetic purity, uniformity, sanitary and phyto-sanitary standards.

3. The planting shall not have been subjected any chemical or bio-physical treatment unless the PPV&FR Authority allows or requests such treatment. If it has been treated, full details of the treatment must be given. The setts with healthy buds shall be carefully packed without damage to the buds.

III. Conduct of tests

1. The minimum duration of DUS tests shall normally be at least at two independent similar growing seasons.

2. The tests shall normally be conducted at two test locations. If any essential characteristics 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 plants or parts of plants could be removed for observation and measurement without prejudicing the other observations on the standing plants until the end of the growing period. Each test plot shall include at least a total of 80 plants. 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 location.

4. Test plot design:

Number of rows	:	4
Row length	:	6 m
Row to row distance	:	90 cm
Plant to plant distance	:	60 cm
Number of replications	:	2
Expected number of plants	:	80

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

6. Additional tests protocols for special purpose (sucrose and fiber content) may 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 for their DUS.

2. All observations for the assessment of Distinctiveness and Stability shall be made on at least 20 culms (stems or canes) from 20 different stools (plants), which shall be divided between 2 replications (10 plants in each replication), unless otherwise indicated (In sugarcane, cane or stem is known as 'culm' and a 'stool' is a cluster of culms derived from a single bud sett of sugarcane, used for vegetative propagation. Botanically, it is analogous to a single plant).

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 0.1% with an acceptance probability of at least 95% shall be applied. In case of a sample size of 80 plants, the number of aberrant plants or parts of plants shall not exceed one.

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

5. All observations on culm (stem) shall be made on fully developed culm preferably primary cane.

6. All observations on the node and internode shall be made at mid height of the fully developed cane.

7. All observations on the leaf blade and leaf sheath shall be made on 3rd or 4th leaf below the 'Top Visible Dewlap' (TVD) leaf.

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 vary only slightly within a variety and which in their various states of expression are fairly evenly distributed across all varieties in the collection are suitable for grouping purposes.

2. The following characteristics shall be used for grouping Sugarcane varieties:
   a) Plant: Growth habit (characteristic 1)
   b) Leaf blade: Curvature (characteristic 6)
   c) Plant: Adherence of leaf sheath (characteristic 8)

3. Grouping characteristics may also be used in the selection of reference varieties to be grown in the trial with candidate varieties.

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 characteristic for the purpose of digital data processing.

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 explanation of the characteristic 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 or figure(s) for clarity and not for the colour variation.

4. A decimal code number in the sixth column of Table of characteristics indicates the optimum stage for observation of each characteristic during the growth and development of plant. The relevant growth stages for assessment of each characteristic corresponding to the codes (days after planting) are given below:

Code	Growth stage
240	End of grand growth stage
300	Maturity stage
360	Harvest stage

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

MG: Measurement by a single observation on 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 on a group of plants or parts of plants
VS: Visual assessment by observation of individual plants or parts of plants

VII. Table of characteristics

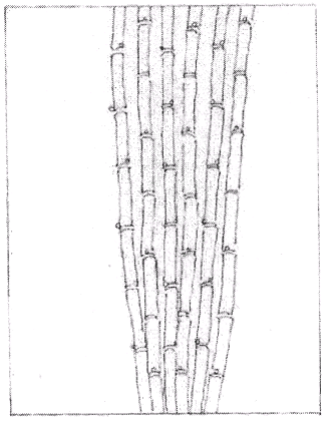
S. No.	Characteristics	States	Note	Example Varieties (tropical / subtropical)	Stage of Obser-vation	Type of Assess-ment
1	**2**	**3**	**4**	**5**	**6**	**7**
1 (*) (+)	Plant: Growth habit	Erect Semi-erect	1 2	Co 87025 / CoLk 8102 Co 86032 / CoH 110	240	VG
2 (*)	Leaf sheath: Hairiness	Absent Sparse Dense	1 3 5	Co 740 CoC 671 Co 7717	240	VS
3 (+)	Leaf sheath: Shape of ligule	Strap-shaped Deltoid Crescent- shaped Arch (bow) shaped	1 2 3 4	CoC 8201 / CoJ 83 Co 94012 Co 97015 / CoS 767 CoV 92103	240	VS
4 (+)	Leaf sheath: Shape of inner auricle	Incipient Deltoid Dentoid Unciform Calcariform Lanceolate Falcate	1 2 3 4 5 6 7	Co 89029 / CoS 767 Co 87271 98R 278 Co 8338 CoA 8401 Co 7318 Co 7805	240	VS
5 (*)	Leaf sheath: Colour of dewlap	Green Greenish - yellow Yellow Yellowish - green Brown Purple	1 2 3 4 5 6	CoG 93076 CoC (SC) 22 CoM 6806 CoA 89081 Co 1101 CoC 671	240	VS
6 (*) (+)	Leaf blade: Curvature	Erect Curved tip Arched	1 2 3	Co 86032 Co 62175 / CoSe 92423 Co 775 / CoJ 64	240	VS
7 (*)	Leaf blade: Width	Narrow (< 3.0 cm) Medium (3.0-5.0 cm) Broad (>5.0 cm)	3 5 7	Co 285 Co 87268 Co 775	240	MS

1	2	3	4	5	6	7
8 (*) (+)	Plant: Adherence of leaf sheath	Weak (self de-trashing) Medium (semi clasping) Strong (tight clasping)	3 5 7	Co 419 / CoS 88230 Co 91010 / CoS 767 CoA 92081 / CoS 797	300	VG
9 (*) (+)	Internode: Colour (Not exposed to sun)	Green (RHS 138-143) Green yellow (RHS 1) Green white (RHS 157) Yellow (RHS 2-11) Yellow green (RHS 145 - 154) Yellow white (RHS 158) Orange white (RHS 159) Greyed green (RHS 193) Greyed yellow (RHS 160)	1 2 3 4 5 6 7 8 9	Co 87044 Co 85017 Co 8013 Co 97015 Co 94012 Co 740 Co 385 Co 87268 Co 87025	300	VS
10 (*) (+)	Internode: Colour (Exposed to sun)	Green yellow group (RHS 1) Yellow green group (RHS 144-154) Yellow group (RHS 3-13, 22) Greyed group (RHS 160-182, 184, 199) Brown group (RHS 200) Purple group (RHS 59-65, 77)	1 2 3 4 5 6	Co 527 Co 87268 Co 86010, 97R 383 Co 86032, Co 91010, Co 508, Co 94012 Co 87025 Co 85002, Co 92020	300	VS
11 (*) (+)	Internode: Diameter	Thin (<2.2 cm) Medium (2.2 – 3.0 cm) Thick (>3.0 cm)	3 5 7	Co 8013 / CoS 8118 Co 86032 / CoS 8436 Co 8371	300	MS
12 (*) (+)	Internode: Shape	Cylindrical Tumescent Bobbin shaped Conoidal Obconoidal Curved	1 2 3 4 5 6	Co 97015 / CoLk 8102 Co 798 / CoJ 83 CoN 91132 / CoLk 7901 Co 89029 CoA 93082 Co 85019	300	VS
13	Internode: Zig zag Alignment	Absent Present	1 9	Co 91010 / CoS 767 Co 87044 / CoSe 95422	300	VG
14	Internode: Growth crack (Split)	Absent Present	1 9	Co 97015 / CoS 767 Co 8021 / CoS 98259	300	VS
15 (*) (+)	Internode: Rind surface appearance	Smooth Corky patches only Ivory marks only Corky patches and ivory marks present	1 2 3 4	Co 87268 CoV 92103 Co 8338 Co 419, Co 86032	300	VS
16 (*)	Internode: Waxiness	Light Medium Heavy	3 5 7	CoC 671 / CoS 767 Co 740 / CoS 8432 Co 94008 / CoS 94270	300	VG

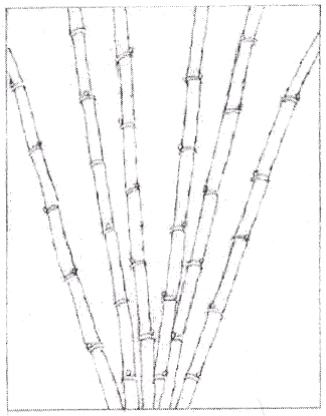
1	2	3	4	5	6	7
17 (+)	Node: Shape of bud	Ovate Obovate Oval Round Pentagonal Rhomboid Rectangular Triangular pointed Beaked	1 2 3 4 5 6 7 8 9	Co 94008 Co 7218 Co 8371 97R 401 Co 8011 CoJaw 270 Co 997 86A 146 CoSnk 05103	300	VG
18	Node: Size of bud (Measured from base of bud to the tip)	Small (6 mm or less) Medium (6-9 mm) Large (9 mm or more)	3 5 7	97V 97 Co 91010 97R 401	300	MS
19 (*)	Node: Bud groove	Absent Shallow Deep	1 3 5	Co 92020 / CoS767 CoC 671 / CoS 96275 Co 86010 / Co 89029	300	VS
20 (*)	Node: Bud cushion (Space between bud base and leaf scar)	Absent Present	1 9	Co 97015 / CoS767 Co 86032 / CoH 92201	300	VS
21	Node: Bud tip in relation to growth ring	Below growth ring Touching the ring Above growth ring	1 3 5	Co 8208 CoC 671 Co 62175	300	VS
22 (+)	Node: Prominence of growth ring	Weak (Not swollen) Strong (Swollen)	1 9	Co 85004 Co 89029	300	VS
23	Node: Width of root band (Opposite to bud)	Narrow Medium Broad	3 5 7	Co 8338 / CoSe 95422 Co 86032 / CoS 767 CoA 90081 / CoS 95270	300	MS
24	Internode: Cross-section	Round Oval	1 2	Co 94012 / CoLk 8001 Co 86032 / CoS 767	360	VS
25	Internode: Pithiness	Absent Present	1 9	Co 85002 / CoS 767 Co 89029 / CoSe 92423	360	VS
26 (+)	Plant: Number of millable canes (NMC) per stool	Low (<3.0) Medium (3.0 – 5.0) High (5.1 – 7.0) Very high (>7.0)	3 5 7 9	Co 85002 / CoS 8436 Co 97015 / CoS 767 Co 94012 / CoS 8118 Co 85004	360	MS
27 (+)	Plant: Cane height	Short (<1.75 m) Medium (1.75-2.5 m) Tall (2.6 – 3.25 m) Very tall (>3.25 m)	3 5 7 9	Co 87271 Co 97015 Co 94012 CoC 773	360	MS

VII.Explanation on the Table of characterstics

Characterstic 1. Plant : Growth habit
Observations made visully on a group of plants (stools) between ground level and 60 cm height inside the plot.

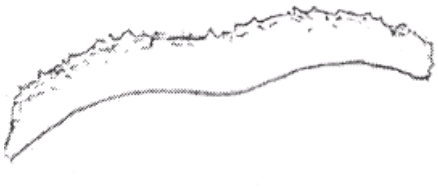


1
Erect



2
Sem-erect

Characterstic 3. Leaf sheath: Shape of ligule



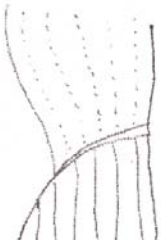
1
Strap-shaped

2
Deltoid

3
Crescent-shaped

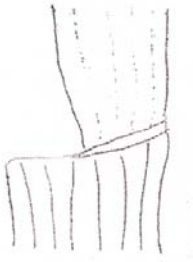
4
Arch(blow) shaped

Characterstic 4. Leaf sheath: Shape of inner auricle

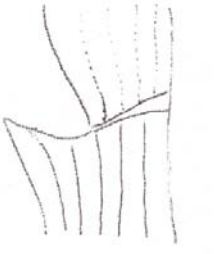


1
Incipient

1
Incipient



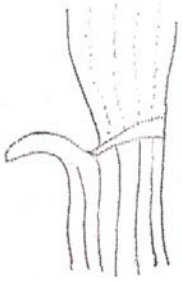
1
Incipient



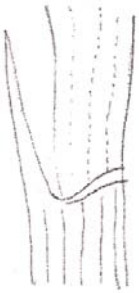
2
Deltoid

3
Dentoid

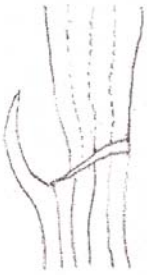
4
Unciform



5
Calcariform



6
Lanceolate



7
Falcate

Characterstic 6. Leaf blade: Curvatiure

1
Erect

2
Curved tip

3
Arched

Charactersitc 8. Plant: Adherence of leaf sheath

Observation shall be recorded by pulling dry leaves and shall be grouped as weak, if leaf sheath is removed completely and very easily, medium, if small part of the leaf sheath remains attached with stem and strong, if leaf sheath is strongly and completely attached with stem.

Charactersitc 9. Internode: Colour not exposed to sun

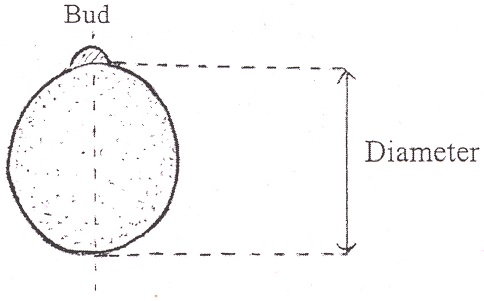
Unexposed colour shall be recorded by removing two lower most green leaves of the stem(after removing the wax with moist cotton) in the middle of the plot.

Charactersitc 10. Internode: Colour exposed to sun

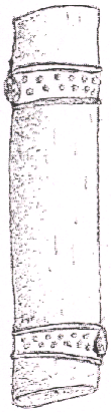
Colour of the stem shall be recorded at fifth internode from the base, two months after de- trashing and removing the wax with moist cotton.

Characterstic 11. Internode:Diameter

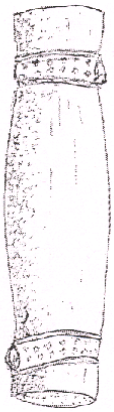
Diameter shall be recorded in centimeter with vernier calipers.The measurement shall be taken at the middle of cane and at the middle of the internode on the axis passing through the bud.



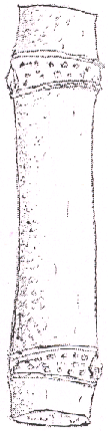
Characterstic 12. Internode: Shape



1
Cylindrical



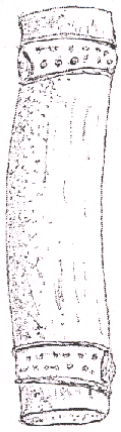
2
Tumescent



3
Bobbin shaped

4
Conoidal

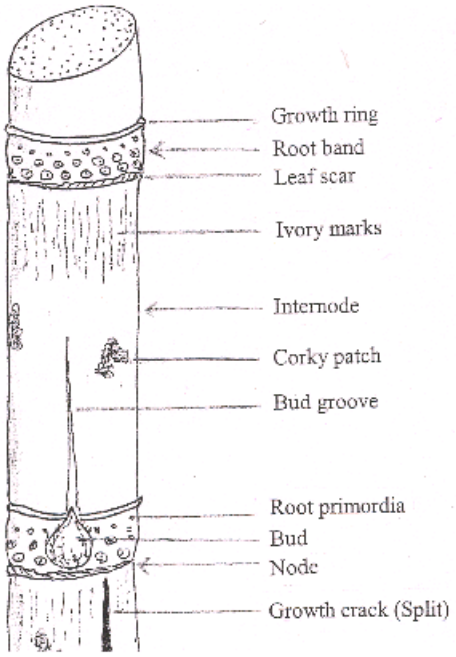
5
Obconoidal



6
Curved

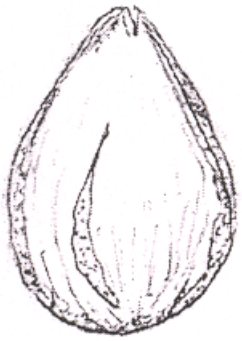
Characterstic 15. Internode: Rind surface appearance

Corky patches (also known as weather marks) are irregualr shaped grey or brown patches on the epidermis.Ivory marks are shallow crevices on the rind below nodes appeaing as short or long thin lines. Also known as corky cracks, imparts rough texture to the rind.

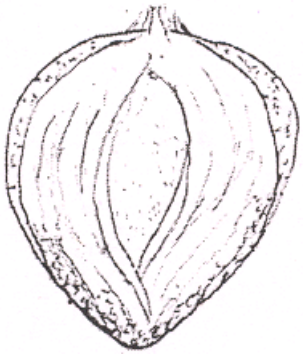


Characterstic 17. Node : Shape of bud

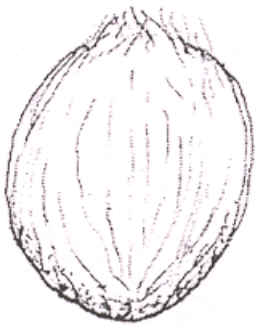
This characterstic shall be observed on the bud at top most joint whose leaf had fully dried and was most reliable.



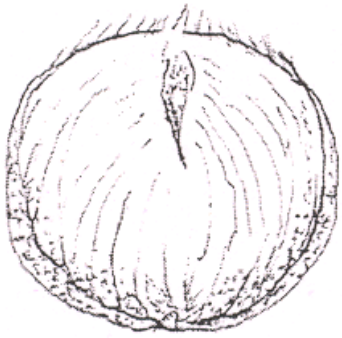
1
Ovate



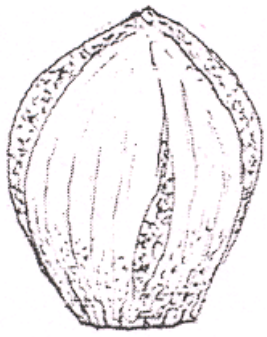
2
Obovate



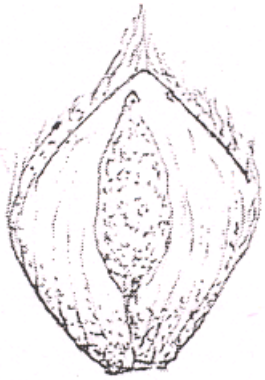
3
Oval



4
Round

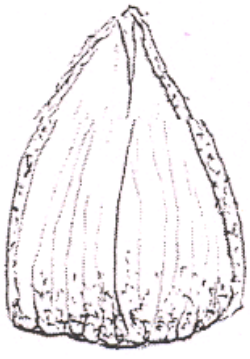


5
Pentagonal

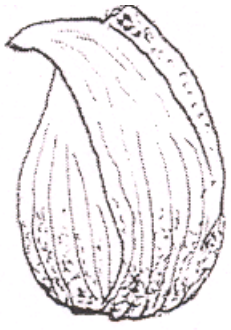


6
Rhomboid

7
Rectangular



8
Triangular pointed



9
Beaked

Characteristic 22. Node: Prominence of growth ring
This is a region just above the root zone and shall be observed at a location opposite to the bud in the middle of cane.

Characteristic 26. Plant: Number of millable canes (NMC) per stool
Fully developed cane with a minimum height of one meter shall be considered as millable cane.

Characteristic 27. Plant: Cane height
Height of the cane shall be recorded between ground level and base of TVD leaf .

IX. Literature

1. Artschwagar, E. (1940). Morphology of the vegetative organs of sugarcane. J.Agric. Res. 60:503-549.
2. Van Dillewijn C.(1952). Botany of Sugarcane. Waltham, Mass**.,** U.S.A. The Chronica Botanica Co.: Book Department.
3. Sugarcane *(Saccharum* L.*)* Guidelines for the conduct of tests for DUS (2005) TG/188/1**,** UPOV, Geneva

X. Working group details

These test guidelines were developed by the National Core Committee in consultation with the Project Coordinator (Sugarcane), IISR, Lucknow; the Nodal Officer, DUS test center, SBI, Coimbatore and Task Force (2/2006) constituted by the PPV&FR Authority.

The Members of the Task Force (2/2006):
Dr. Y. S. Nerkar (Chairman)
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Dr. D. M. Hegde
Dr. P. S. Pathak
Dr. H. S. Sen
Dr. R. K.Chowdhury
Dr. S. S. Banga
Dr. A. K. Singh
Dr. P. S. Bhatnagar

Nodal Officers:
SBI, Coimbatore (Tropical): Dr. V. A. Amalraj, Principal Scientist
IISR, Lucknow (Sub - tropical): Dr. J. Singh, Senior Scientist

XI. DUS test centres:

DUS test centres (Tropical varieties)	**DUS test centres (Sub-tropical varieties)**
Sugarcane Breeding Institute, Coimbatore – 641007,Tamilnadu.	Indian Institute of Sugarcane Research, Lucknow – 226002, Uttar Pradesh.
Sugarcane Breeding Institute, Research Centre, Agali – 678581, Kerala.	Sugarcane Breeding Institute, Regional Centre, Karnal – 132001, Haryana.