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

Betelvine
(*Piper betle L.*)

Protection of Plant varieties and Farmer's Rights Authority

(PPV & FRA)

Government of India

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Betelvine(*Piper betle L.)*

I. Subject

These test guidelines shall apply to all varieties and hybrids of Betelvine (*Piper betle L.*) grown under open system of cultivation and closed (*Boroj*) system of cultivation.

The open system of cultivation under natural condition is practiced in North Eastern and Southern states. Betelvine is grown with arecanut (*Areca catechu* L.) and *Sesbania grandiflora* as support crop for the vine.

Boroj is an artificially erected closed hut structure, the main frame work of which is made of bamboo poles to a height of 2m. Its sides and roof are made of locally available materials like jute stick, straw, grass banana leaf etc. For support of the vine, jute sticks or sliced bamboo sticks or reeds are used.

II. Planting material required

The protection of Plant Varieties and Farmer's Rights Authority (PPV & FRA) shall decide when, where and in what quantity and quality the planting material is required for testing a variety denomination applied for registration under the protection of Plant Varieties and Farmer's Rights 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 number of planting materials (rooted cuttings) to be supplied by the applicant shall be 15 in open system 30 for closed system of cultivation.

1. The planting material supplied shall be healthy, not lacking in vigor or affected by any pest or diseases as well as nutrient deficiency. The age of the rooted cutting from the terminal shoots shall be 3 months from the date of planting in the polythene bags [20cm x 10cm size with soil mixture (1:1:1 soil, FYM and sand)]. The rooted cutting shall be of minimum height of 25 cm.

2. The plant material should not have undergone any treatment which would affect the expression of the characteristics of the variety. The planting material shall not have undergone any chemical or bio- physical treatment unless the Competent Authority allow or request such treatments. If it has been treated, full details of the treatment must be given.

III. Conduct of tests

1. The minimum duration of DUS tests shall be two crop years from one year after planting from same plants or till the observations recorded on leaves from plagiotropic branches. For the purpose of these test guidelines, crop years include continuous leaf harvestable years (Leaves from plagiotropic shoot in open system of cultivation and leaves from orthotropic shoot in closed system).

2. The test shall be conducted at one place suitable to its growing systems. If any essential characteristics of the candidate variety are not expressed for visual observation at this location, the variety shall be tested for further examinations at another test site or under special test protocol on expressed request of the applicant.

3. The field test shall be carried out under favoring normal growth and expression of all test characteristics. In particular, a satisfactory crop must be produced in two crop years. As a minimum, each test shall include fifteen (in open system) thirty vines (in closed system) which shall be divided between two or more replicates.

4. **Test plot design**

Open system of cultivation

Standard : Under Areca nut

Duration : 2 years

Spacing : 2.7/2.7m

Number of replications : 3

Plants/ replication : 5

Standard : Under Sesbania

Duration : 2 years

Spacing : 100 cm x 20 cm

Number of replications : 3

Plants/ replication : 5

Closed system of cultivation

Standard : Bamboo sticks

Duration : 2 years

Spacing : 70 cm x 10 cm

Number of replications : 3

Plants/ replication : 10

5. Additional test protocols for special tests established by the PPV & FR Authority.

IV. Methods and observation

1. The characteristics described in the Table of characteristics (section VII) shall be used for testing of varieties and hybrids for their DUS.

2. Unless otherwise indicated, all observations determined by measurement or counting shall be made on five vines or parts of five vines.

3. All the leaf characters shall be recorded on harvestable mature leaves of orthotropic shoot which are present on 8^{th} to 10^{th} node from the tip under closed system of cultivation. All the leaf characters shall be recorded on the 2^{nd} /3^{rd} (harvestable) leaf from plagiotropic shoot in open system of cultivation.

4. All observations shall be taken only from the one year old established vines.

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 evenly distributed across all varieties in the collection, are suitable for grouping purposes.

2. The following characteristics shall be used for grouping of Betelvine varieties.

   i. Plant: Orthotropic stem colour (Characteristic 2)
   ii. Leaf: Orthotropic leaf l/b ratio (Characteristic 7)
   iii. .Leaf: Orthotropic leaf petiole length (Characteristic 8
   iv. Leaf: Proximity of basal lobes of orthotropic leaf (Characteristic 16)
   v. Leaf: Leaf lamina colour (orthotropic or plagiotropic) (characteristic 4 &19)
   vi. Leaf: Plagiotropic leaf l/b ratio (characteristic 23)
   vii. Plant: Sex of the plant (characteristic 24)
   viii. Female inflorescence: Color (characteristic 26)
   ix. Male inflorescence: Length (characteristic 28)

VI. Characteristics and symbols

1. To assess Distinctiveness, uniformity and stability, the characteristics and their states are given in the table of characteristics (Section VII) shall be used. The characters shall be recorded in open and closed system of cultivation as specified in the section VIII.
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 state 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 phenomenal characteristic or by the environmental conditions of the testing region. Under such exceptional situation, adequate explanation shall be provided.

(*):Closed condition(Boroj)

(**): Open condition

(*, **): open/closed condition

(+) See explanation 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 or figure(s) for clarity and not for the colour variation.

4. A 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 code numbers are described below:

a) Observations should be made on fully established one year old vines.

b) Observations should be made on harvestable mature leaves on orthotropic shoots

c) Observations should be made on harvestable mature leaves on plagiotropic shoots

d) Observations should be made on six months after the vine produces plagiotrpic shoots.

e) Observations should be made during flowering period

5.Type of assessment of characteristics indicated in column seven 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 observation of individual plant or parts of plants

VII. Table of characteristics

S. No	Characteristics	States	Note	Example variety	Stage of observation	Type of assessment
1	**2**	**3**	**4**	**5**	**6**	**7**
1 (+)	Plant:Adventitious root production (Closed)	Few (<5) Medium (5-10) Many (> 10)	3 5 7	Halisahar Sanchi Kali Bangla DogapanSada	a	MS
2 (*) (+)	Plant: Orthotropic stem colour (Closed)	Light Green Green Moderately Green Dark Green	1 2 3 4	Swarna Kapoori CARI-6 Ghanegette Gangarampur Sanchi	a	VG
3. (+)	Plant: Orthotropic stem internodal length (cm) (Closed/open)	Very short(<4) Short (4- 6.0) Medium (6.0-8.0) Long (> 8.0)	1 3 5 7	- Ghanagette Kutki Bangala, Godi Bangla CARI-2 ,CARI 6	a	MS
4. (*) (+)	Leaf: Orthotropic leaf lamina color (Closed/open)	Light Green (RHS Yellow green group 144A,144 B, 146A) Green (RHS Green group 137A,B,N137A,B,C, 143A,B,C) Dark Green (139A)	1 2 3	Swarna Kapoori Ghanegette ,Godi Bangla CARI-2	b	VG
5 (+)	Leaf: Orthotropic leaf lamina length (l) (cm) (Closed)	Very short (<7.0) Short (7.0- 11.5) Medium(11.5-14.5) Long (> 14.50)	1 3 5 7	- Kadwa Kali Bangla Kutki Bangala	b	MS

6 (+)	Leaf: Orthotropic leaf breadth (b) (cm) (Closed)	Narrow (<9.50) Medium (9.50-12.50) Broad (> 12.50)	3 5 7	Kadwa Lakshman Kari Bangla	b	MS
7 (*) (+)	Leaf: Orthotropic leaf l/b ratio (Closed)	Low (<1.30) Medium (1.30-1.50) High (> 1.50)	3 5 7	Lakshman Meetha-2 Gangarampur Sanchi	b	MS
8 (*) (+)	Leaf: Orthotropic leaf petiole length (cm) (Closed)	Short (< 6.0) Medium (6.0-8.0) Long (> 8.0)	3 5 7	Simurali Sanchi Lakshman Ghanagette	b	MS
9 (+)	Leaf: Orthotropic leaf thickness (µm) (Closed)	Thin (<190) Medium (190-230) Thick (> 230)	3 5 7	 Kalbaghini Bagherhat	b	MS
10 (+)	Leaf: Depth of sinus orthotropic leaf (cm) (Closed)	Shallow (<0.60) Medium (0.60-1.20) Deep (>1.20)	3 5 7	Kalbaghini SimuraliBhabna Kari Bangla	b	MS
11 (+)	Leaf: Width of orthotropic leaf lobe (cm) (Closed)	Short (<3.50) Medium (3.50-5.00) Long (> 5.00)	3 5 7	Kalbaghini Ghanagette Kari Bangla,	b	MS
12 (*) (+)	Leaf: Depth of sinus/ width of lobe of orthotropic leaf (Closed)	Entire or Slightly lobed (< 0.15) Moderately lobed (0.15- 0.25) Deeply lobed (>0.25)	1 2 3	SimuraliSanchi Kalbaghini Ghanagette	b	MS

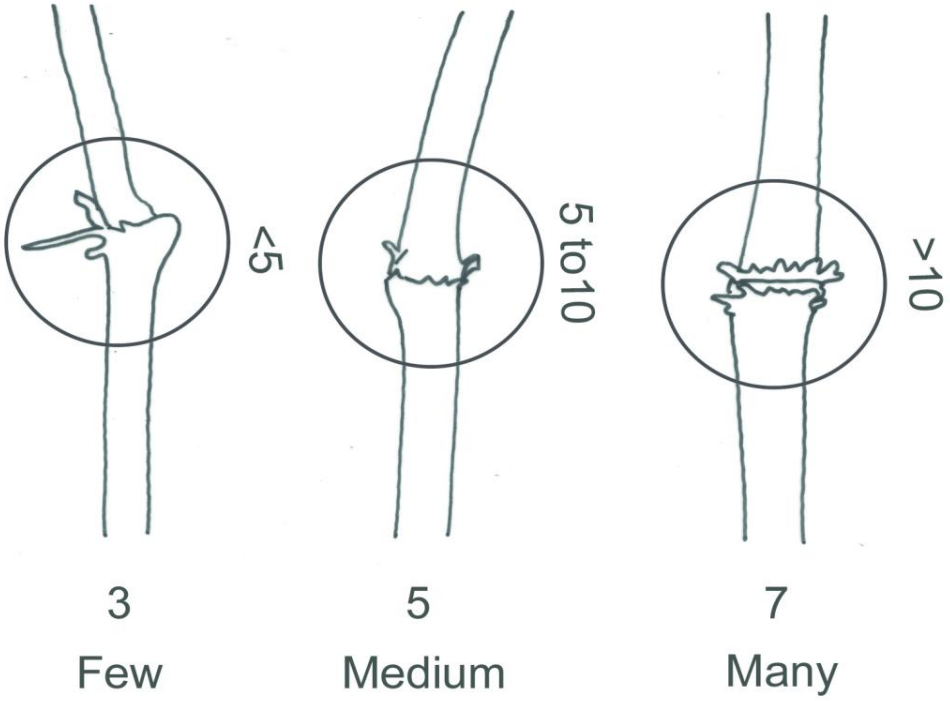
13 (+)	Leaf: Leaf lamina orientation along the midrib (Closed)	Flat lamina V-shaped lamina	1 2	Kapoori Pedacheppali CARI-2	b	VG
14 (+)	Leaf: Orthotropic leaf apex shape (Open)	Acuminate Acute	1 2	Sirugamani-1 Banavalli	b	VG
15 (+)	Leaf: Orthotropic leaf texture (Open)	Coriaceous Membranaceous	1 2	Banavalli Maghai	b	VG
16 (*) (+)	Leaf: Proximity of basal lobes of orthotropic leaf (Closed)	Overlapped Close Separate	1 2 3	Ghanagette Bankura Bangla CARI-2	b	VG
17 (+)	Number of plagiotropic shoots (No/m) (Open)	Low (<3) Medium (3 -6) High (>6)	3 5 7	- Bangla(UP) Sirugamani 1	d	MS
18 (+)	Plant: Plagiotropic stem colour (Open)	Light Green (RHS Yellow green group 144B,144C) Green (RHS Green group 137B,138A)	1 2	Swarna Kapoori CARI-6	d	VG
19 (*) (+)	Leaf: Plagiotropic leaf lamina colour (Open)	Light Green (RHS Yellow green group 144A,144 B, 146A) Green	1 2	Swarna Kapoori Godi Bangla	c	VG

		(RHS Green group 137A,B,N137A,B,C, 143A,B,C) Dark Green (139A)	 3	 CARI-6		
20 (+)	Leaf: Plagiotropic leaf lamina shape (open)	Elliptic Ovate oblong Ovate	1 2 3	SwarnaKapoori Sirugamani-I Godi Bangla	c	VG
21 (+)	Leaf:Plagiotropic leaf apex Shape. (open)	Acuminate Acute	1 2	Swarna Kapoori Banavalli	c	VG
22 (+)	Leaf:Plagiotropic leaf texture. (open)	Coriaceous Membranaceous	1 2	Banavalli Swarna Kapoori	c	VG
23 (*) (+)	Leaf:Plagiotropic leaf l/b ratio (open)	Low (<1.5) Medium (1.5 to 2.0) High (>2.0)	3 5 7	Godi Bangla Sirugamani-1 CARI-6	c	MS
24 (*) (+)	Plant:Sex of the plant (open)	Female Male Hermaphrodite	1 2 3	HalisaharSanchi Swarna Kapoori --	e	VG
25 (+)	Flowering habit (open)	Shy flowering Moderateflowering Profuse flowering	3 5 7	Maghai Halisahar Sanchi Swarna Kapoori	e	VG
26 (*) (+)	Female inflorescence: Colour (open)	Beige Yellow	2 4	Sirugamani 1 HalisaharSanchi	e	VG
27 (+)	Female inflorescence: length (cm) (open)	Short (<2.5cm) Medium (2.5 to 4cm) Long (>4cm)	3 5 7	Maghai Sirugamani-1 Halisahar Sanchi	e	MS

28 (*) (+)	Male inflorescence: length (cm) (open)	Short(<7) Medium (7.0 to 10 cm) Long (>10cm)	3 5 7	- Swarna Kapoori, IIHR BV96-1	e	MS
29 (+)	Number of inflorescence /Plagiotropic branch (open)	Low (<2.0) Medium (2.0 to 4.0) High (>4)	3 5 7	Maghai Halisahar Sanchi CARI-6	e	MS
30 (+)	Leaf: Taste (Closed/open)	Sweet Low Pungent Moderate Pungent Highly Pungent	3 5 7 9	Meetha Pan Swarna Kapoori Halisahar Sanchi Karapaku	b & c	VG
31 (+)	Eugenol Content (%) (Closed/open)	Absent (0) Low (0-10) Moderate (10-20) High (20-40) Very High (>40)	1 2 3 4 5	- Godi Bangla Halisahar Sanchi Swarna Kapoori -	c	MG

VIII. Explanation for the Table of characteristics

Characteristic 1.Plant: Adventitious root production



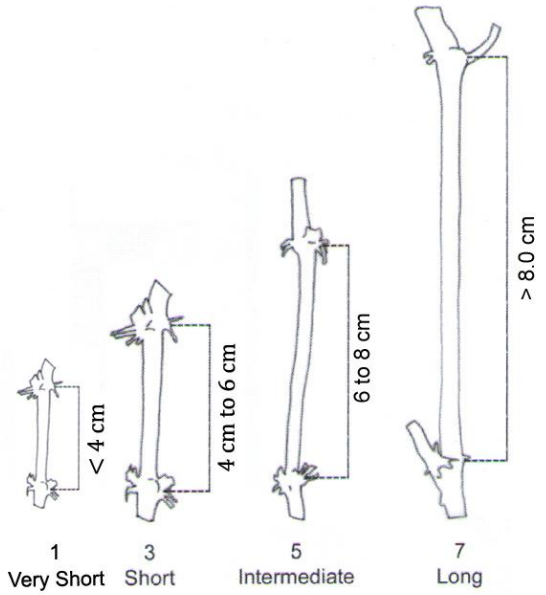
Adventitious roots shall be counted at 4th, 5th& 6th nodes from the tip of the orthotropic shoot (mean of 3nodes) from five vines.

Characteristics 2.Plant: Orthotropic stem colour

The Orthotropic stem colour shall be noted at 3rd and 4th nodes from tip of the vine. The visual assessment of the appearance shall be noted.

Characteristic 3. Plant: Orthotropic Stem internodal length (cm)

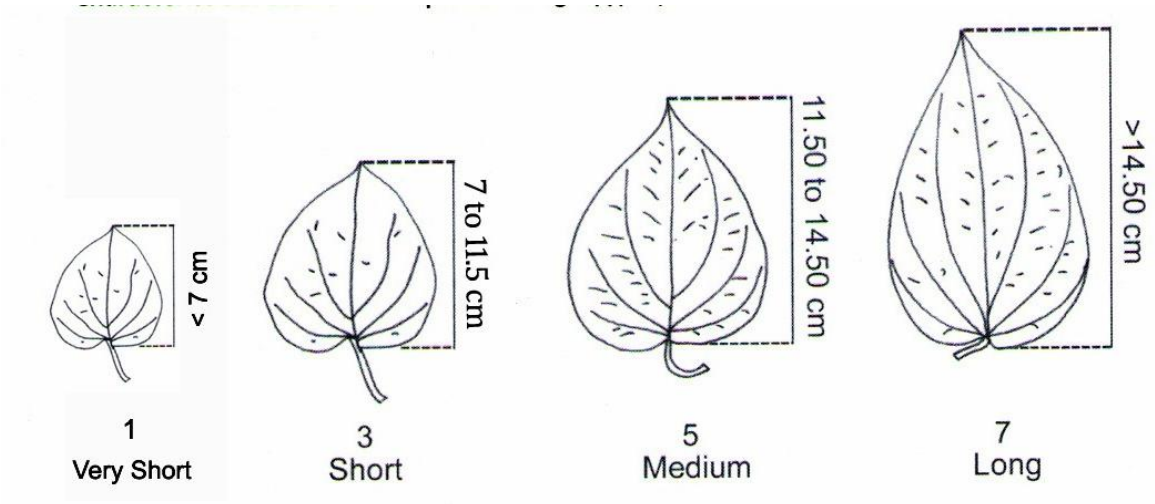
Orthotropic stem internodal length shall be measured from 5th to 8th internodes from the tip of the orthotropic stem as mean of 3 nodes from five vines.



Characteristic 4.Leaf: Orthotropic leaf lamina Colour

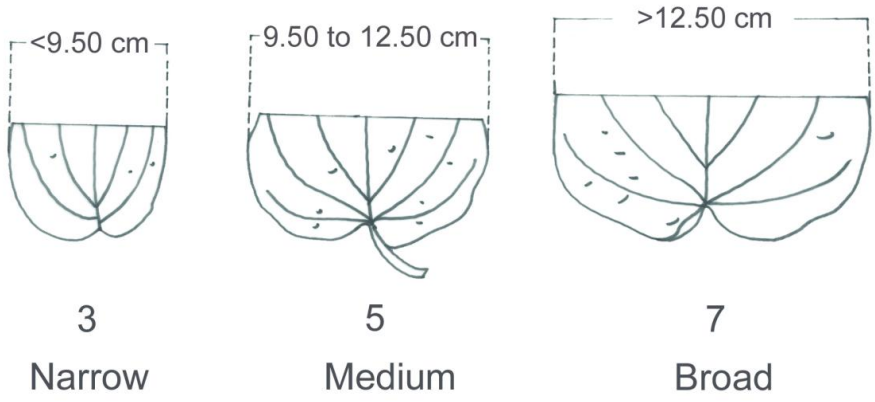
Orthotropic leaf colour shall be observed on harvestable leaves from orthotropic shoot

Characteristic 5. Leaf: Orthotropic leaf lamina length (l) (cm)



Leaf length will be measured as distance between point of attachment of lamina with petiole and the tip of the leaf from 25 mature leaves of five randomly selected vines.

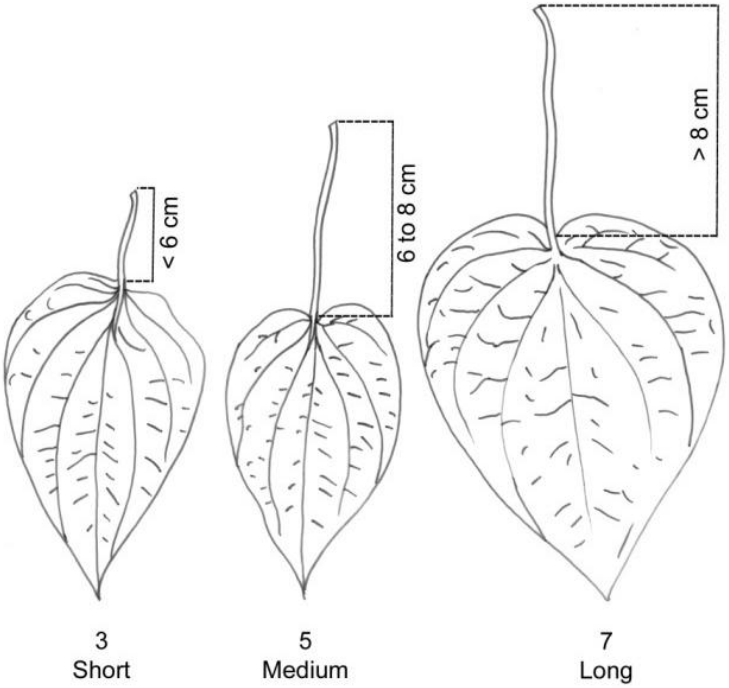
Characteristic 6.Leaf: Orthotropic leaf breadth (b) (cm)



Leaf width will be measured as maximum distance between two lateral margins with the help of a scale from 25 mature leaves of five randomly selected vines.

Characteristic 7.Leaf: Orthotropic leaf l/b ratio: Orthotropic leaf l/b ratio will be calculated as length of leaf divided by width of leaf from 25 observations from five vines.

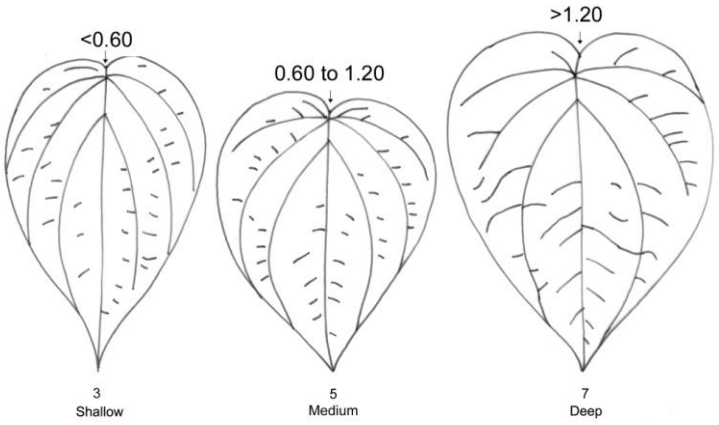
Characteristic 8.Leaf: Orthotropic leaf petiole length (cm)



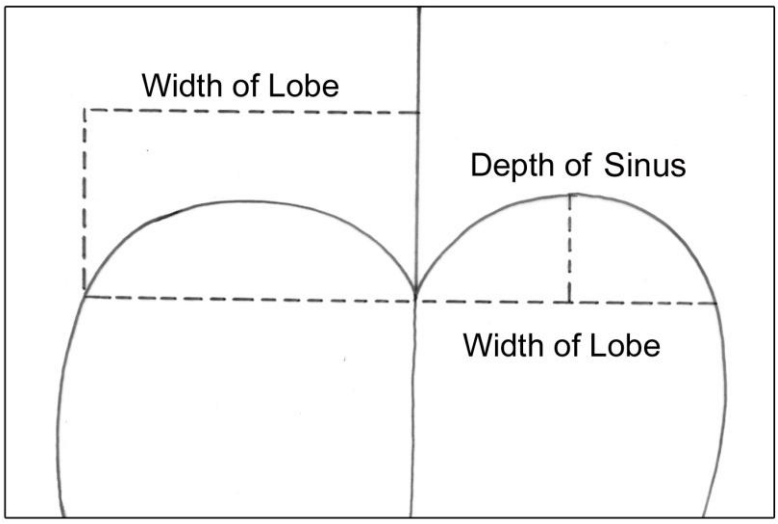
Leaf petiole length will be measured as distance between points of attachment of the petiole with stem and lamina from 25 mature leaves of five randomly selected vines.

Characteristic 9.Leaf: Orthotropic leaf thickness (µm): Orthotropic leaf thickness will be measured from 25 mature leaves of five randomly selected vines with the help of stereo microscope.

Characteristic 10&11.Leaf: Depth of sinus & width of orthotropic leaf lobe (cm)



Depth of sinus will be calculated as length of leaf including lobe subtracted by leaf length (Leaf length from midrib) and measured from 25 mature leaves of five randomly selected vines.



Leaf lobe width will be measured from one side, left or right.

Characteristic 12. Leaf: Depth of sinus/ width of lobe of orthotropic leaf

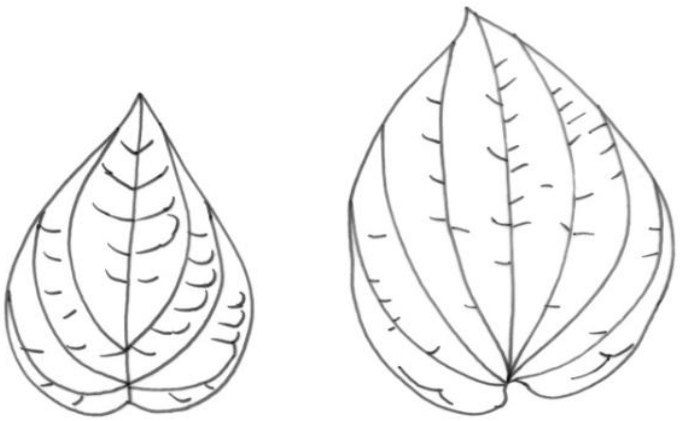
Ratio of depth of sinus to width of leaf lobe will be calculated. According to ratios, three categories will be made as follows:

Entire or slightly lobed (< 0.15)

Moderately lobed (0.15- 0.25)

Deeply lobed (>0.25)

Characteristic 13. Leaf: Leaf lamina orientation along the midrib



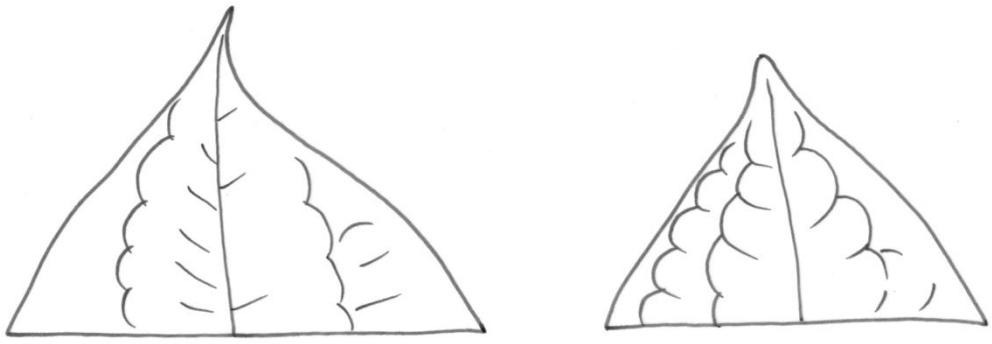
1
Flat Lamina

2
V-Shaped Lamina

Flat lamina: both sides of the lamina are at same plane.

V-shaped lamina: two sides of lamina form an inner angle at the midrib.

Characteristic 14.Leaf: Orthotropic leaf apex shape



1
Acuminate

2
Acute

Orthotropic leaf apex shape is assessed from the harvestable leaves from orthotropic stem as given below

Acuminate- The margins between the apex and 0.75L is concave, curving toward the center of the leaf, or is convex basally and concave apically

Acute-the margin between the apex and 0.75L curves away from the center of the leaf

(L=Leaf length)

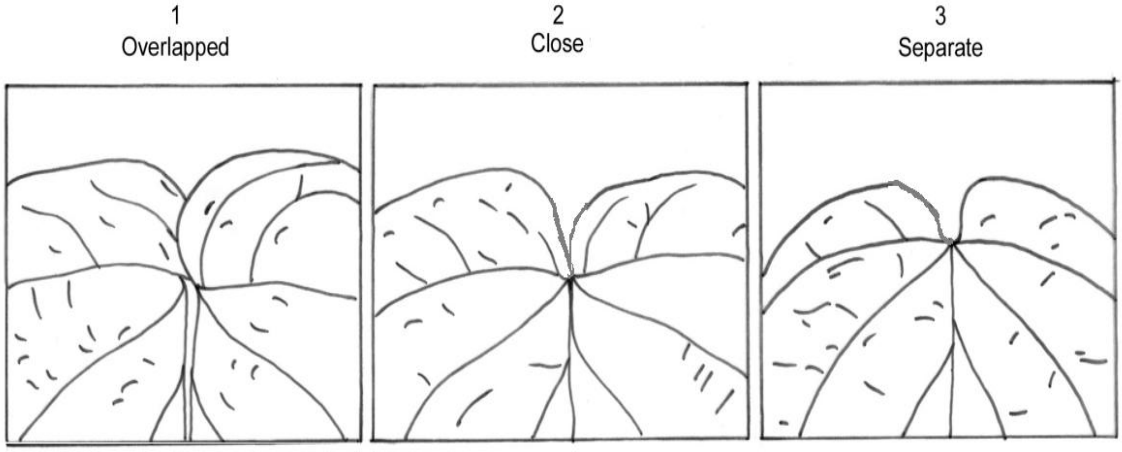
Characteristic 15.Leaf: Orthotropic leaf texture

Orthotropic leaf texture is observed on the harvestable leaves from orthotropic Shoot.

Coriaceous-Leaf texture is thick and leathery

Membranaceous-Leaf texture is thin

Characteristic 16. Leaf: Proximity of basal lobes of orthotropic leaf



On the basis of relative distance between basal lobes of leaf, three categories will be made as follows:

Lobes overlapped: when lobes are physically overlapping each other near the point of attachment of lamina and petiole.

Close to overlap: when lobes are physically very close but not overlapping.

Separate: when lobes are sufficiently apart from each other

The visual assessment of the appearance shall be noted.

Characteristic 17. Number of plagiotropic shoots (No/m)

Number of plagiotropic shoots in one meter length shall be counted on the orthotropic stem leaving 30 cm from the base in five vines.

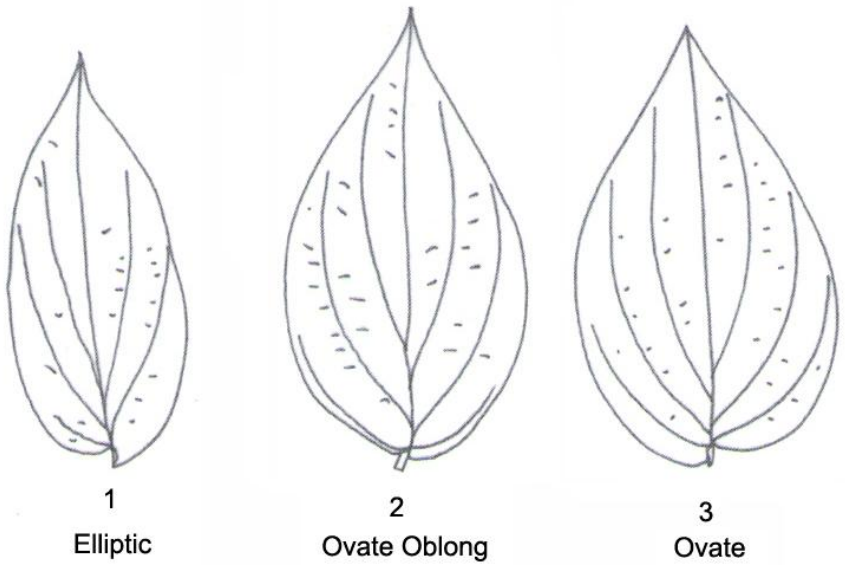
Characteristic 18.Plant: Plagiotropic stem colour

Plagiotropic stem colour shall be assessed on terminal portion between 3rd& 4th node.

Characteristic 19.Leaf: Plagiotropic leaf lamina colour

Plagiotropic leaf colour shall be assessed on harvestable leaves of plagiotropic shoot

Characteristic 20.Leaf: Plagiotropic leaf lamina shape



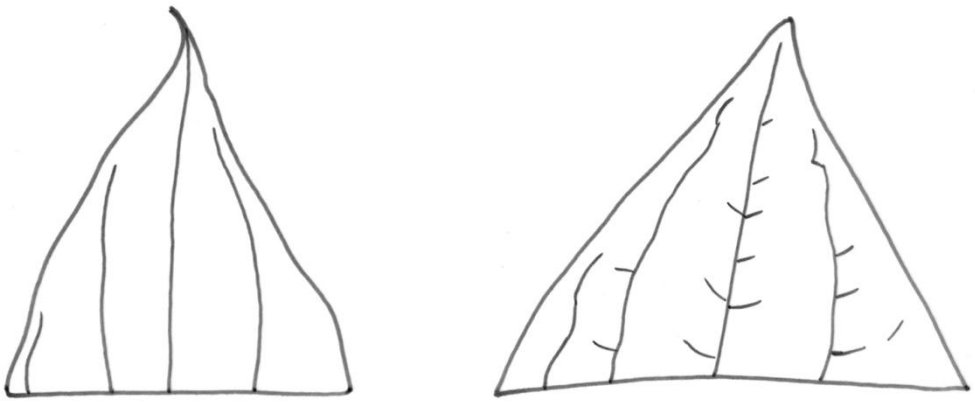
Plagiotropic leaf lamina shape shall be observed from the harvestable leaves of plagiotropic shoots as described below

Elliptic- The widest part of the leaf is on an axis in the middle fifth of the long axis of the leaf

Ovate oblong- The widest part of the leaf is on an axis in the middle fifth of the long axis of the leaf but ovate in shape

Ovate-The widest part of the leaf is on axis in the basal 2/5 of the leaf.

Characteristic 21. Leaf: Plagiotropic leaf apex shape



1 Acuminate

2 Acute

Plagiotropic leaf apex Shape shall be assessed on the harvestable leaves of plagiotropic shoot

Acuminate- The margins between the apex and 0.75L is concave, curving toward the center of the leaf, or is convex basally and concave apically

Acute-the margin between the apex and 0.75L curves away from the center of the leaf (L= Leaf Length)

Characteristic 22. Leaf: Plagiotropic leaf texture.

Plagiotropic leaf texture shall be observed from the harvestable leaves of plagiotropic shoot

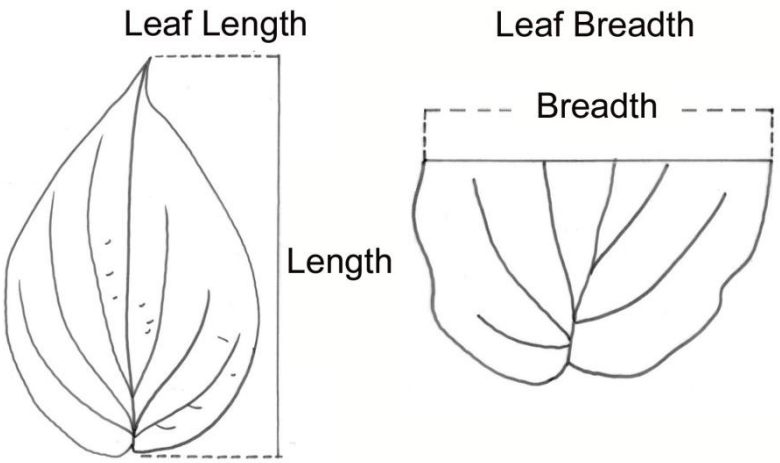
Coriaceous-Leaf texture is thick and leathery

Membranaceous-Leaf texture is thin

Characteristic 23.Leaf: Plagiotropic leaf l/b ratio

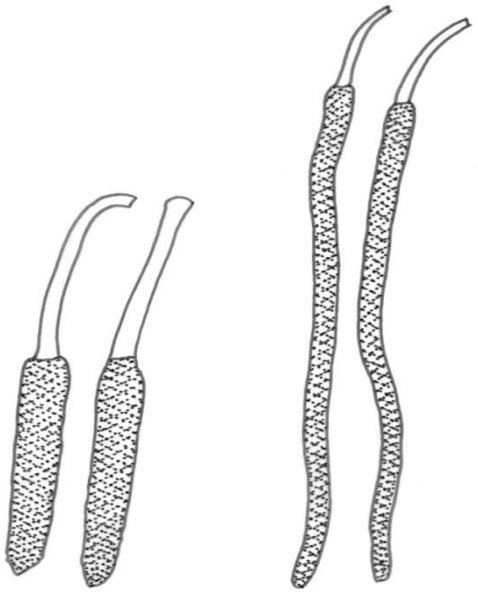
Plagiotropic leaf length(l):Leaf length will be measured as distance between point of attachment of lamina with petiole and the tip of the leaf from 25 harvestable plagiotropic leaves of five randomly selected vines.

Plagiotropic leaf breadth (b): Maximum leaf width will be measured as maximum distance between two lateral margins from 25 harvestable plagiotropic leaves of five randomly selected vines.



Plagiotropic leaf l/b ratio is calculated by dividing length /breadth of the leaf (average of 25 leaves from five vines)

Characteristic 24.Plant: Sex of the plant



1
Female

2
Male

Sex of the vine shall be assessed from inflorescences borne on plagiotropic shoots
Female- vine with pistillate flowers only.
Male - vine with staminate flowers only.
Hermaphrodite-vine with hermaphrodite flowers only

Characteristic 25: Flowering Habit

Flowering Habit shall be assessed on duration of flowering and number of inflorescences per Plagiotropic shoot

Shy flowering: Flowering is observed for 1-2 months or less

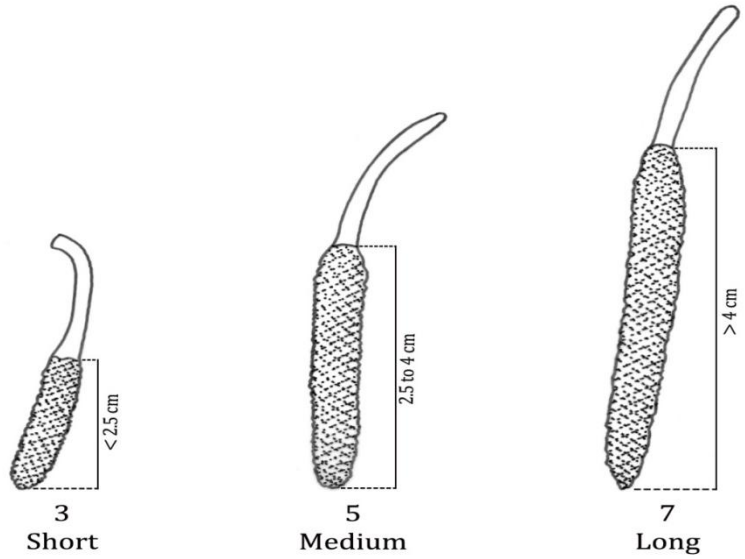
Moderate flowering: Flowering observed for 4-5 months

Profuse flowering: Flowering is observed throughout the year

Characteristic 26.Female inflorescence : colour

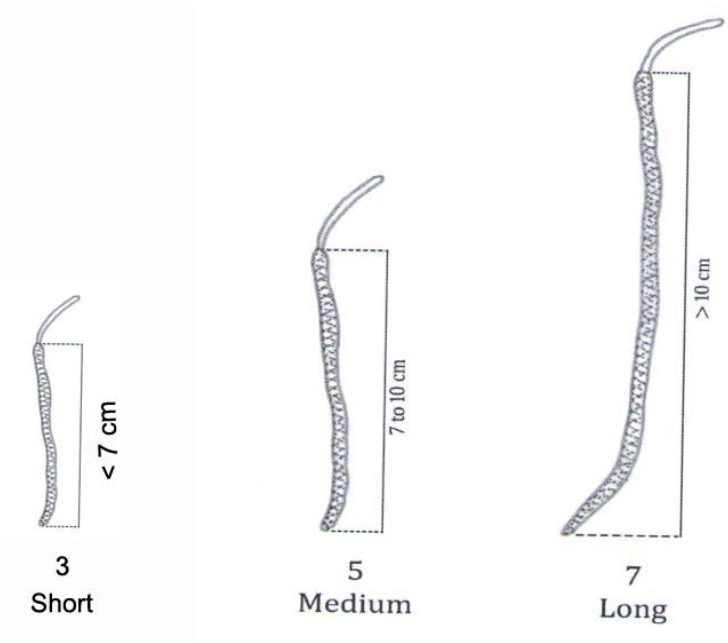
Inflorescence colour shall be observed on inflorescences found on plagiotropic shoots of female varieties

Characteristic 27.Female inflorescence: length (cm)



Inflorescence length shall be measured on inflorescences on plagiotropic shoots at full bloom stage in female varieties (average of 10 female inflorescences)

Characteristic 28. Male inflorescence: length (cm)



Inflorescence length shall be measured on Inflorescences on plagiotropic shoots at full bloom stage in male varieties (average of observations on 10 male inflorescences)

Characteristic 29: Number of inflorescence /plagiotropic branch

Number of inflorescence /plagiotropic branch shall be counted on plagiotropic shoots (average of 15 plagiotropic shoots from five vines)

Characteristic 30: Leaf: Taste

Orthotropic and Plagiotropic leaf taste shall be assessed on harvestable leaves of Orthotropic & plagiotropic shoot

Characterstic 31: Eugenol content:

Fresh harvestable leaves essential oil is extracted through hydro distillation and Eugenol content analyzed with GC-MS/MS.

IX. Literature

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X. **Working group details**

The test guidelines developed by the task force **(09/2014)** constituted by the PPV & FR Authority for **Betelvine** with consultation by Indian Institute of Horticultural Research (IIHR) Bangalore and Bidhan Chandra KrishiViswavidyalaya (BCKV), Kalyani, West Bengal and Technical inputs also provided by the PPV & FR Authority and nodal officer.

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7. **Dr. Ravi Prakash** **Member**
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XI. DUS testing centers

Nodal DUS test centre	Co nodal DUS Test Center
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