

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

**NEEM**

***(Azadirachta indica A. Juss..)***



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

## **NEEM (*Azadirachta indica* A. Juss.)**

### **I. Subject**

These Test Guidelines shall apply to all clonally propagated varieties of Neem (*Azadirachta indica* A. Juss.)

### **II. Planting Materials Required**

1. The Protection of Plant Varieties and Farmers Rights Authority (PPV & FRA) shall decide on the quantity and quality of the plant material required for testing the variety and when and where it is to be delivered for registration under the Protection of Plant Varieties and Farmers Rights (PPV & FRA) Act, 2001.
2. Applicants submitting such plant 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. Clonally propagated plant materials of 60 cm height from collar to the apical tip are required for DUS testing. The plants must have fully developed root system. The planting material should be supplied in 15 cm x 25 cm container.
4. The minimum number of planting material to be supplied by the applicant or his nominee during June-July shall be 40 clonally rooted plants.
5. The age of the plants shall be 6 months while submitting for testing.
6. The plant material should be visibly healthy, not lacking in vigour or affected by any pests or diseases.
7. The plant material should not have undergone any treatment, which would affect the expression of the characteristics of the variety, unless the competent authorities allow or request such treatment. If it has been treated, full details of the treatment must be given.

### **III. Conduct of Tests**

#### *Duration of test*

The minimum duration of DUS tests shall normally up to two independent flowering cycles.

### *Testing Place*

The tests shall normally be conducted at two locations. If any essential characteristics of the candidate variety are 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 expression of interest of the applicant.

### *Conditions for Conducting the Examination*

The tests should be carried out under conditions ensuring satisfactory growth for the expression of the relevant characteristics of the variety and for the conduct of the examination.

### *Test Design*

The design of the tests should be such that plants or parts of plants may be removed for measurement or counting without prejudice to the observations which must be made up to the end of the growing cycle.

### **Test Plot Design**

No. of rows	: one
Row to row distance	: 5 m
Plant to plant distance	: 5 m
No. of plants per replication	: 6
No. of replications	: 3

The test plot will be surrounded by one guard row. Additional test protocol for special purpose shall be established by the PPV & FR Authority.

### ***On-site DUS testing***

- a. On-site testing shall be conducted at the places specified by the applicant.
- b. The age of the trees at on-site shall be minimum of 10 years with the potentiality of exhibiting all morphological and reproductive characters.
- c. A trial with minimum of 1 tree shall be considered for on-site testing to provide provisional registration of variety.
- d. Once provisional registration with minimum of 1 tree is approved, the registrant must supply 40 clonally propagated planting materials from mother tree (Registered Tree) for

regular DUS Testing. The registration will be granted only on the successful testing of clonal progeny as per the procedures laid down in the DUS testing guidelines by the PPV & FR Authority.

- e. The trees must be healthy and free from pest and disease and raised under standard management practices.
- f. The Expert Committee constituted by the PPV & FRA in consultation with the DUS Centre shall be authorized to inspect on-site testing and recording of the appropriate characters.

#### **IV. Methods and Observations**

- a. The characteristics described in the Table of characteristics shall be used for testing of varieties for their DUS (Section VII).
- b. The assessment of Distinctiveness and Stability of all observations shall be made on 6 plants or parts taken each of 6 plants, which will be equally divided among 3 replications (2 plants per replication).
- c. The assessment of Uniformity of characteristics shall be made in 6 plants per replication, with an acceptance probability of at least 95%. The maximum number of off-type allowed would be 1 in 18 plants.
- d. All observations of leaf shall be made in mature leaves at middle of the crown in the middle third of the youngest shoots not showing signs of active growth. A sample of 10 leaves per tree (representing all four directions of the tree) shall be taken for morphometric characterization.
- e. The branchlet, flower and fruit characteristics should be evaluated from 10 samples each collected from nine trees. Samples should be collected from the longest primary branch in the mid portion of the crown.
- f. Observations on the inflorescences should be made at the time of peak flowering on inflorescences borne on typical shoots from the exposed regions of the tree.
- g. Observations on mature fruit should be recorded when the fruit is ready for harvesting.
- h. Observations on seeds should be made on 10 typical seeds taken from a minimum sample size of 50 fully developed seeds.
- i. For the assessment of all colour characteristics, the latest Royal Horticultural Society (RHS) colour chart shall be used.

## **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 the varieties in the collection are suitable for grouping purpose.
2. The following characteristics shall be used for grouping of Neem varieties:
  - a. Leaflet shape (Characteristics 2.5)
  - b. Flower: Petals shape (Characteristics 3.1)
  - c. Fruit shape (Characteristics 4.3)
  - d. Fruit: Shape of apex (Characteristics 4.4)
  - e. Seed: Shape (Characteristics 5.3)
  - f. Seed: Tip (Characteristics 5.4)

## **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 given for each state of expression for different characteristics for the purpose of electronic data processing.
3. Legend:
  - i. (\*) 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 the environmental conditions of the testing region. Under such exceptional situation, adequate explanation shall be provided.
  - ii. (+) See Explanation on the Table of Characteristics in Section VIII. It is to be noted that for certain characteristics, plant parts on which observations to be taken are given in the explanation or figure(s) for clarity and not the colour variation.
4. A decimal code in the sixth column of the Table of Characteristics indicates the stage for the observation of each characteristic during the growth and development of the variety. The relevant growth stages corresponding to the decimal code number are described below.

Code	Examination of Characteristics	Stage of Observation
1.	<b>Tree crown character</b>	<ul style="list-style-type: none"> <li>a. The observation on the tree habit was made when the entire tree is found with foliage.</li> <li>b. Observations on the tree habit were made on mature trees with a fully developed trunk and crown with complete foliage of atleast 5 years of age capable of exhibiting all morphological and reproductive characters.</li> </ul>
2.	<b>Leaf character</b>	<ul style="list-style-type: none"> <li>a. All the observations on leaf were made on fully developed leaves from admits of vigorous current season shoots occupying the peripheral/circumference of tree crown.</li> <li>b. All observations for length and width on the mature leaf and leaflets were made on the central part of leaf/leaflet.</li> <li>c. All observations for length of petiole and rachis were made on the mature leaf.</li> </ul>
3.	<b>Inflorescence character</b>	<ul style="list-style-type: none"> <li>a. Observations on the flowers were taken from the fully developed inflorescence at the beginning of anther dehiscence and also at the time of full flowering of the tree.</li> <li>d. Observations on the flowers were made on the second and subsequent flowers present in the inflorescence stage as described in the item 3a.</li> <li>b. Observations on the flower colour were made at peak flowering stage under natural day light condition.</li> </ul>
4.	<b>Fruit character</b>	<ul style="list-style-type: none"> <li>a. All fruits for observation were taken from periphery of the tree and fruit misformed as a result of clustering were not sampled.</li> <li>b. Observations on the fruits were made on 10 typical fruits taken from a minimum sample size of 50 fruits at the time of full maturity.</li> <li>c. Observations on the fruit shape were presented as they appear in nature; nevertheless shape is to be observed in direction from the base (stalk end) to the top.</li> <li>d. All observations for length and width on the mature fruit were made on the longest and broadest portion of the fruit respectively.</li> </ul>
5.	<b>Seed character</b>	<ul style="list-style-type: none"> <li>a. All observations on the seeds were made on the fresh matured seed in fruits at full maturity stage.</li> <li>b. Observations on the seed length/width were made on 10 typical seeds taken from a</li> </ul>

		<p>minimum sample size of 50 fully developed seeds.</p> <p>c. Observations on the seed colour were made under natural day light condition.</p> <p>d. Observation on the seed shape was made on fully mature seeds.</p> <p>e. Observation on oil and azadirachtin content were made on kernel basis from 50 typical seeds taken from a minimum sample size of 500 fully developed seeds.</p>
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5. Characteristics containing the following key in the first column of the table of characteristics shall be examined as indicated below

QL: Qualitative characteristics

QN: Quantitative characteristics

PQL: Pseudo - qualitative characteristics

6. Type of assessment of characteristics indicated in column seven of Table the 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 plants

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

## VII. Table of Characteristics

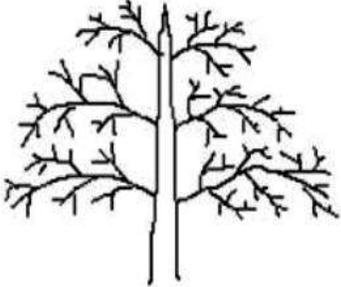
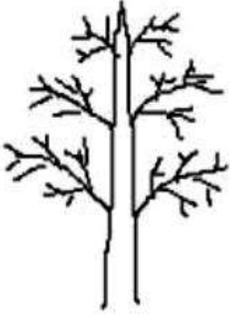
S.No.	Characteristics	State	Note	Example Source	Stage of observation	Type of assessment
1 (* )	Tree habit (PQL)	Spreading	1	Mettupalayam	1b	VG
		Semi-upright	2	Bhavanisagar		
		Upright	3	Sirumugai		
2 (* )	Leaflet length (QN)	Short (<5.0 cm)	3	Mulli	2b	MG
		Medium (5.0 -10 cm)	5	Dharmapuri		
		Long (>10.0 cm)	7	Kovilpatti		
3 (* )	Leaflet width (QN)	Narrow (<1.5 cm)	3	Annur, Pillur	2b	MG
		Medium (1.5-3.0 cm)	5	Dharmapuri		

		Broad (>3.0 cm)	7	Pudukottai		
4 (* )	Petiole: Length (QN)	Very short (<4.0 cm)	1	Mulli	2c	MG
		Short (4.1 - 8.0 cm)	3	Karamadai		
		Medium (8.1-12.0 cm)	5	Pudukottai		
		Long (>12.0 cm)	7	Kovilpatti		
5 (* )	Inter Leaflet: Rachis Length (QN)	Very short (<2.0 cm)	1	Mulli	2c	MG
		Short (2.0 - 3.0 cm)	3	Karamadai		
		Medium (3.1- 4.0 cm)	5	Virudhunagar		
		Long (4.1 - 5.0 cm)	7	Pudukottai		
		Very long (>5.0 cm)	9	Kovilpatti		
6 (+ )	Leaflet shape (PQL)	Falcate	1	Thiruchirapalli	2a	VG
		Lanceolate	2	Sivagangai		
7 (+ )	Leaf margin: Serration (QL)	Serrate	1	Bhavanisagar	2a	VS
		Dentate	9	Mulli		
8 (+ )	Petals shape (PQL)	Spatulate	1	Mettupalayam	3a	VG
		Obovate	2	Salem		
9 (* )	Fruit length (QN)	Short (<1.5 cm)	3	Mulli	4b	MG
		Medium (1.5-2.0 cm)	5	Bhavani		
		Long (>2.0 cm)	7	Pudukottai		
10 (* )	Fruit width (QN)	Narrow (<1.0 cm)	3	Mulli	4b	MG
		Medium (1.0-1.5 cm)	5	Palladam		
		Broad (>1.5 cm)	7	Kovilpatti		
11 (+ )	Fruit shape (PQL)	Oblong	1	Coimbatore	4c	VG
		Oval	2	Annur, Madurai		
		Globular	3	Erode, Musri		
12 (+ )	Fruit: Apex (PQL)	Round	1	Annur	4c	VG
		Acute	2	Kavindhapadi		
13 (+ )	Fruit: Stalk cavity (QL)	Absent	1	Gobi	4a	VG
		Present	9	Arupukottai		
14 (+ )	Presence of neck (QL)	Absent	1	Arupukottai	4a	VG
		Present	9	Paiyur		

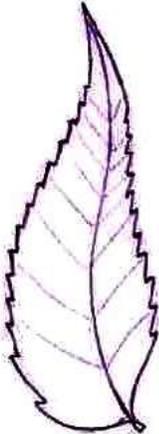
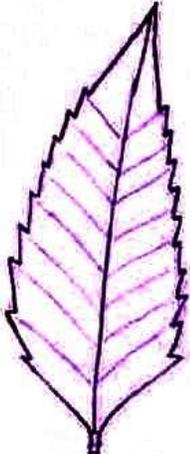
15 (+)	Presence of fruit shoulder <b>(QL)</b>	Absent	1	Bhavani	4a	VG
		Present	9	Athani		
16 (* )	Seed length <b>(QN)</b>	Short (<1.0 cm)	3	Mulli	5b	MG
		Medium (1.0-1.5 cm)	5	Athani		
		Long (>1.5 cm)	7	Manamadurai		
17 (* )	Seed width <b>(QN)</b>	Narrow (<0.5 cm)	3	Mulli	5b	MG
		Medium (0.5-0.8 cm)	5	Dharapuram		
		Broad (>0.8 cm)	7	Manamadurai		
18 (+)	Seed shape <b>(PQL)</b>	Oblong	1	Karamadai	5a	VG
		Ovate	2	Athani		
19 (+)	Seed tip shape <b>(PQL)</b>	Acute	1	Dindigul	5a	VG
		Obtuse	2	Mettupalayam		
20 (* )	Oil content <b>(QN)</b>	Low (<36%)	3	MTP - 2	5e	MG
		Medium (36% - 41%)	5	MDU - 1 MDU - 2 MDU - 6		
		High (41% - 45%)	7	APK - 1 APK - 2 MDU - 3 MDU - 5 MDU - 7 MDU - 8		
		Very High (> 45%)	9	F15L26T22 (Jhansi)		
21 (* )	Azadirachtin content <b>(QN)</b>	Low (<0.30%)	3	MDU -3	5e	MG
		Medium (0.30% -0.60%)	5	MTP - 1 APK - 4 APK - 6 MDU - 2 MDU - 6 MDU - 8		
		High (>0.60%)	7	MTP - 2		

## VIII. Explanations on the Table of Characteristics

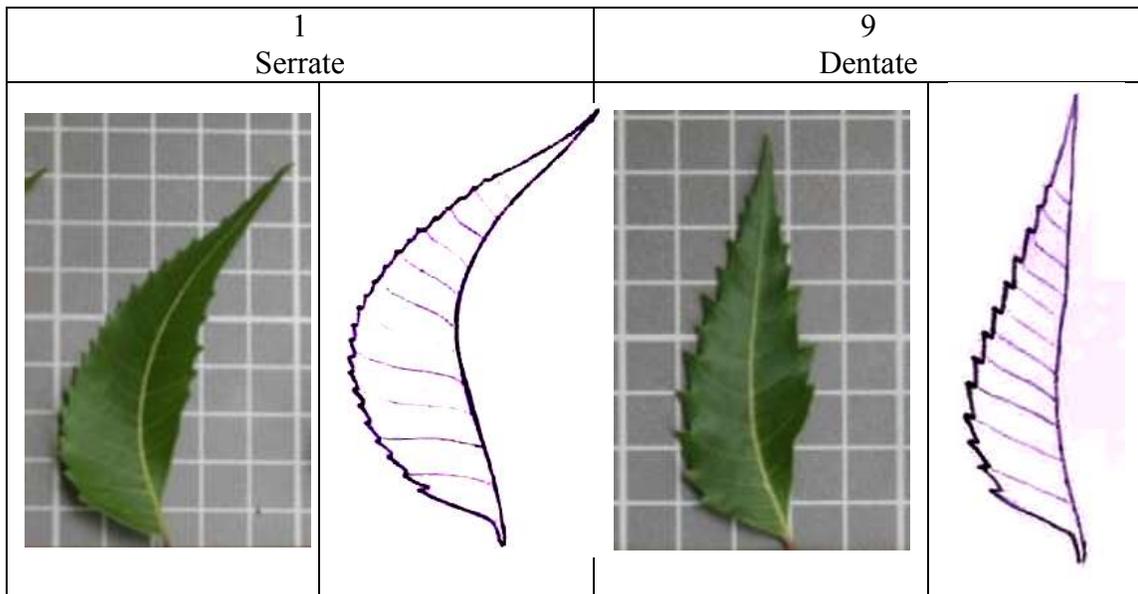
### Characteristic 1: Tree habit

1 Spreading	2 Semi-upright	3 Upright
		
		

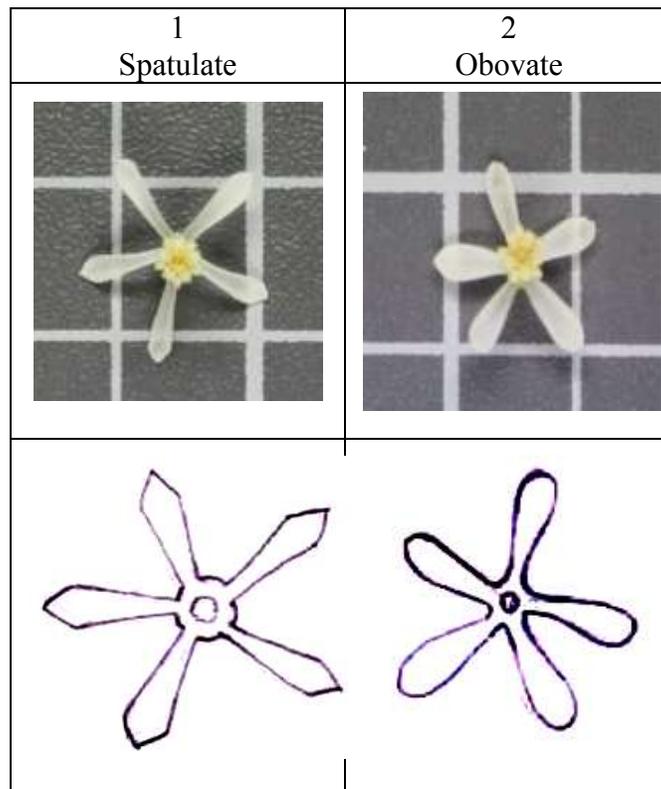
### Characteristic 6: Leaflet shape

1 Falcate		2 Lanceolate	
			

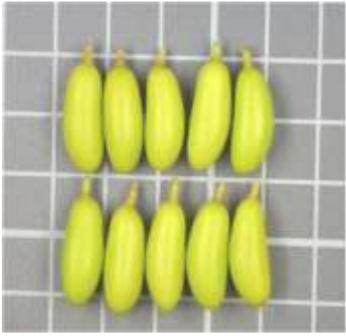
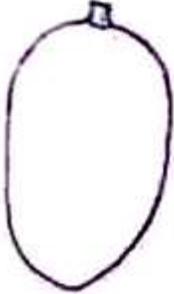
**Characteristic 7: Leaf margin**



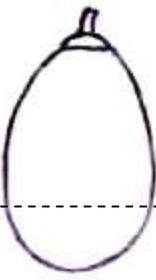
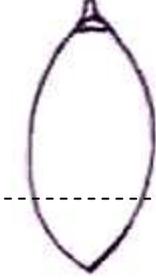
**Characteristic 8: Petals shape**



**Characteristic 11: Fruit shape**

1 Oblong	2 Oval	3 Globular
		
		

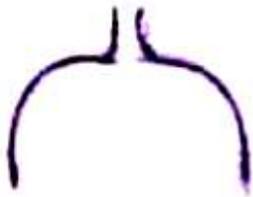
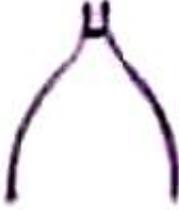
**Characteristic 12: Fruit: Apex**

1 Round	2 Acute
 	 

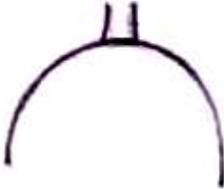
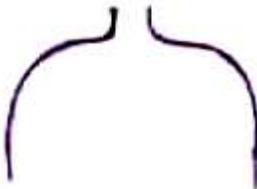
**Characteristic 13: Fruit: Stalk cavity**

1 Absent	9 Present
	
	

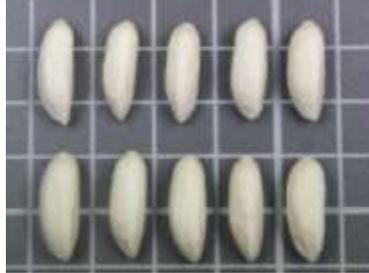
**Characteristic 14: Presence of fruit neck**

1 Absent	9 Present
	
	

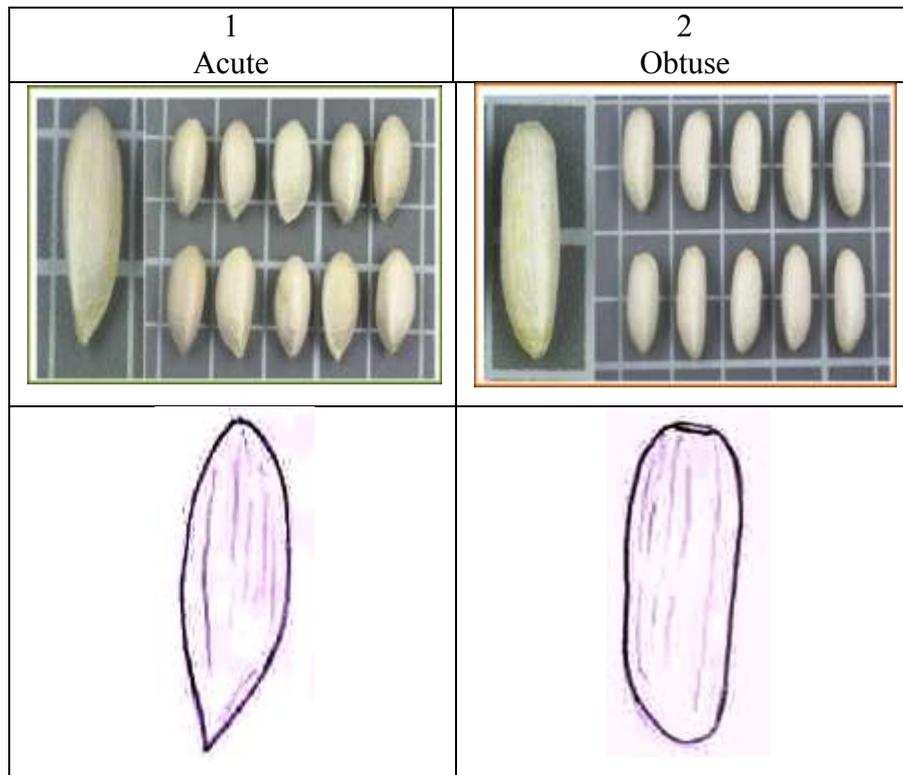
**Characteristic 15: Presence of fruit shoulder**

1 Absent	9 Present
	
	

**Characteristic 18: Seed shape**

1 Oblong	2 Ovate
	
	

### Characteristic 19: Seed tip shape



### IX. Working Group Details:

The Test Guidelines developed by Forest College and Research Institute, Tamil Nadu Agricultural University, Mettupalayam was approved by the Task Force (03/2014) constituted by the PPV & FR Authority.

### The Members of the Task Force (03/2014)

Dr.B.Gurudev Singh	Chairman
Dr. Balakrishna Gowda	Member
Dr.K.Kumaran	Member
Dr.A.Balasubramanian	Member
Dr.Ravi Prakash	Member Secretary
Dr.N.A.Prakash	Special Invitee

### **Nodal Persons**

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### **X. DUS testing centre**

<b>NODAL CENTRE</b>	<b>CO-NODAL CENTRE</b>
Forest College and Research Institute, Tamil Nadu Agricultural University, Mettupalayam, Coimbatore (Dt), Tamil Nadu.	