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

# Walnut

(Juglans regia L.)



Protection of Plant Varieties and Farmer's Rights Authority (PPV & FRA) MOA, Government of India, New Delhi

#### WALNUT (Juglans regia L.)

#### I. Subject

These test guidelines shall apply to all varieties of Walnut (Juglans regia L.)

#### **II. Material 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. 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. As a minimum the applicant need to submit 10 grafted or budded plants of walnut on rootstock for each centre.
- 2. The plant material supplied should be visibly healthy, not lacking in vigour, nor affected by any important pest or disease.
- 3. 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

- 1. The minimum duration of the DUS tests shall normally be at least for two fruiting season in different years. Tests shall be conducted at least at two places.
- 2. 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. Each test should include total of 6 trees. In particular, it is essential that the trees produce a satisfactory crop of fruit in each of the two growing seasons.

#### Test plot 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. The additional test protocol for special purpose if any may be established by PPV & FRA.

1	Locations	:	Two
2	No. of replications	:	Three
3	Treatment unit	:	Two trees per replication (total 6 plants /location)
4	Spacing	:	3 x 3m

#### IV. Methods and observations

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

- 1. For the assessment of Distinctiveness and Stability, observation shall be made on 6 plants or 18 parts taken from each of 6 plants with the exception of the observation on nut and kernel which should be made on at least 20 nuts. In the case of parts of plants, the number to be taken from each of the plant should be three.
- 2. For the assessment of uniformity a population standard of 1% with an acceptance probability of at least 95% should be applied. In the case of a sample size of 6 plants, the maximum number of off-types allowed would be 1.
- 3. All observations on the tree and the branches should be made during dormancy. Observations on the mature fruit / nut should be recorded when fruit is ready for harvesting at packing tissue turning brown.
- 4. The time of staminate and pistillate flowering should be observed when 10% of flowers have opened (at dehiscence of pollen or at full development of stigmas)
- 5. All observations on the leaf should be made on fully developed leaves of the middle third of current season's shoot
- 6. Time of maturity should be recorded at 50% fruits, turns their packing tissue brown
- 7. All observations on the nut should exclude the pericarp and should be made on physiologically mature nuts immediately after harvest.
- 8. All observations on the kernel should be made when the moisture is about 8 percent.
- 9. Type of assessment of characteristics indicated in column 7 of table of characteristics is as follows.
  - a) MG: Measurement by a single observation of a group of plants or parts of plants
  - b) MS: Measurement by a single observation of individual plants or parts of plant
  - c) VG: Visual assessments by a single observation of a group of plants or parts of plants
  - d) VS: Visual assessments by a single observation of individual plants or parts of plant

#### 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 purpose.
- 2. The following characteristics are recommended for grouping of varieties
  - a. Tree growth habit
  - b. Bearing habit
  - c. Leaflet margin
  - d. Hull dehiscence
  - e. Nut shape

#### 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
- (\*) 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 preceding phonological characteristics or by the environmental conditions of the testing region. Under such exceptional situation, adequate explanation shall be provided.
- (+) 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 the color variation.
- 4. A code number in the sixth column of Table-VII of characteristics indicates the optimum stage for the observation of each characteristic during growth and development of plant. The relevant growth stages corresponding to these code numbers are described below:
  - a. All observations on the tree vigour and the branch should be made in winter during dormant conditions. Observation should be made at central third of shoot
  - b. Observations on the leaf which should be made from average of 10 fully expanded represented leaves of current season shoot. Do not select leaves that are abnormal due to pruning and excessive vigor and measured from the base of petiole to the tip of terminal leaflet.
  - c. Observations should be made when more than 10 percent staminate and pistillate flowers are open and well ahead of the first flush of pistillate flowers. Peak bloom dates are usually when about half the catkins and pistillate are fully opened and receptive and half are yet to be opened. Avoid reporting aberrant conditions such as a single, unopened catkin remaining after pollen shedding has ceased or a bloom which is receptive
  - d. All observations on the nut should exclude the pericarp and should be made on the physiological ripe nuts immediate after harvest. Observations are taken when nuts are harvestable .Take a random sample which is representative of entire tree (in the format DDMMYYYY). Time of maturity should be recorded at 50% fruits turns their packing tissue brown.

S. No.	Characteristic s	States	Notes	Varieties characterised	Stages of observation	Type of assessment
1	2	3	4	5	6	7
1.	Tree vigour (cm)	Low ( < 50) Intermediate	3	Opex Caulchery, Tutle, CITH-W-3	а	VG
		(50-100) High	5 7	Sulaiman, Cheinovo, Franquette, CITH-W-2, CITH-W-4, CITH-W-6 Hamdan, Nugget, CITH-W-1,		
	T	(>100)		CITH-W-7		
2. (+)	Tree: Growth habit	Erect Semi erect	3 5	CITH-W-12, CITH-W-54 Cheinovo, Nugget, Sulaiman, Franquette, CITH-W-1, CITH-W-2, CITH-W-6, CITH-W-10	a	VG
		Spreading	7	Hamdan, Opex Caulchery, CITH- W-3, CITH-W-5		
3.	Tree:	Sparse	3	CITH-W-16		
	Density of branches	Intermediate	5	Cheinovo, Sulaiman, Nugget, Franquette, Turtle, CITH-W-2, CITH-W-3, CITH-W-5, CITH-W-6	a	VG
		Dense	7	Hamdan, Opex Caulchery, CITH- W-1, CITH-W-4		
4. (*) (+)	Bearing habit	Terminal	1	Opex Caulchery, Sulaiman, Hamdan, Cheinovo, CITH-W-1, CITH-W-2	С	VG
( )		Lateral	9			
5.	Leaf: Leaflet length (cm)	Short (< 10)	3	Franquette, Tutle, CITH-W-2, CITH-W-3, CITH-W-5		
		Medium (10-15)	5	Cheinovo, CITH-W-7, CITH-W-9	b	MG
		Long (>15)	7	Nugget, Hamdan, Opex Caulchery, Sulaiman, CITH-W-1, CITH-W-4		
6. (*)	Leaf: Leaflet shape	Narrow elliptic	1	CITH-W-4,CITH-W-8,CITH-W-10	b	VG
(+)		Elliptic	2	Franquette, Opex Caulchery, CITH- W-5, CITH-W-7, CITH-W-8, CITH-W-9,		
		Broad elliptic	3	Tutle, Nugget, Sulaiman, Cheinovo, Hamdan. CITH-W-1, CITH-W-6		
7. (*) (+)	Leaf:Leaflet margin	Entire	3	Opex Caulchery, Tutle, Hamdan, Nugget, Cheinovo, Sulaiman, CITH-W-1, CITH-W-2, CITH-W-3	b	VG
		Serrate	5	CITH-W-77		
		Dentate	7	Franquette		
8.	Leaf: Leaflet colour	Light green Green	3	CITH-W-36, CITH-W-45 Hamdan, Nugget, Franquette,	b	VG
			_	CITH-W-4, CITH-W-6,CITH-W-7	U	۰U
		Dark green	7	Tutle , Cheinovo, Opex Caulchery, Sulaiman, CITH-W-1, CITH-W-2, CITH-W-3		
		Purplish	9			
9.	Leaf: Rachis colour	Green	3	Tutle, Hamdan. Nugget, Sulaiman CITH-W-1, CITH-W-2, CITH-W-5		
		Yellow	5	CITH-W-4,CITH-W-6, CITH-W-7	b	VG

		Red	7	Opex Caulchery, Cheinovo, CITH- W-50		
10.	Leaf:Leaflet	Few	3	Opex Caulchery, Tutle		
	rachis persistance	Intermediate	5	Nugget, Sulaiman, Hamdan, CITH- W-2	b	VG
		Many	7	Franquette, CITH-W-1, CITH-W-3, CITH-W-5		
11.	Time of leaf	Early	3	Nugget, Cheinovo, CITH-W-18		
	fall	Mid	5	Opex Caulchery, Hamdan, CITH- W-1, CITH-W-2	b	MG
		Late	7	CITH-W-4, CITH-W-5		
12.	Shoot pubescence	Glabrous	1	Cheinovo, CITH-W-3, CITH-W-7, CITH-W-9		
		Slighty pubescent	2	Hamdan, Sulaiman, CITH-W-1, CITH-W-10	b	VG
		Pubescent	3	CITH-W-2, CITH-W-6, CITH-W-5		
13.	Shoot colour	Green	3			
		Brown	5	Opex Caulchery, Tutle, Hamdan, Nugget, Cheinovo, Sulaiman, Franquette, CITH-W-2, CITH-W-3, CITH-W-4, CITH-W-5	b	VG
		Dark Brown	7			
14. (*)	Dichogamy	Protandrous	3	Franquette, Opex Caulchery, CITH- W-4, CITH-W-7, CITH-W-8, CITH-W-10	с	VG
		Protogynous	5	Tutle, Cheinovo, Nugget, Sulaiman, Hamdan, CITH-W-1, CITH-W-2, CITH-W-3		
		Homogamous	7			
15.	Flower :	Early	3	Sulaiman, CITH-W-1, CITH-W-2		
	Initiation of	Mid	5	Opex Caulchery, CITH-W-4		
	10% Female flowering	Late	7	Franquette, CITH-W-5		
16. (*)	Flower: Number of	Few		Hamdan, CITH-W-5, CITH-W-17, CITH-W-4		
	male catkins percluser	Intermediate	5	Franquette, CITH-W-3, CITH-W-7, CITH-W-8, CITH-W-10	С	VG
		Many	7	Nugget, Cheinovo, Opex Caulchery, Tutle, Sulaiman, CITH- W-2, CITH-W-4, CITH-W-6, CITH-W-7, CITH-W-9		
17. (*)	Flower: Number of	Low (< 2)	3	Sulaiman, Cheinovo, Hamdan, CITH-W-7		
	female flowers per cluster	Medium (2-4)	5	Nugget, Opex Caulchery, Franquette, CITH-W-2, CITH-W-3, CITH-W-4, CITH-W-1	с	MG
		High(> 4)	7	CITH-W-11, CITH-W-43		
18.	Stigma colour	Green	3	Hamdan, Sulaiman, CITH-W-1, CITH-W-6, CITH-W-7, CITH-W-8	с	VG
		Yellow	5	CITH-W-27, CITH-W-33, CITH- W-34,		
		Red	7	Tutle, CITH-W-38, CITH-W-48, CITH-W-31		

19.	Hull	Non –	3	CITH-W-34, CITH-W-35, CITH-		
(*)	dehiscence :	dehiscent		W-36		
. ,	Туре	Partly	5	CITH-W-20, CITH-W-27, CITH-		
		dehiscent	_	W-32	d	VG
		Dehiscent	7	Opex Caulchery, Tutle, Hamdan, Nugget, Cheinovo, Sulaiman Franquette, CITH-W-1, CITH-W-2, CITH-W-3, CITH-W-4		
20.	Time of	Early	3	CITH-W-53, CITH-W-61		
	maturity 50% hull	Medium	5	Opex Caulchery, CITH-W-4, CITH-W-11	d	MG
	dehiscent from 1 <sup>st</sup> Jan	Late	7	CITH-W-45,CITH-W-36, CITH- W-65		
21. (*)	Nut shape	Round	1	Sulaiman, Opex Caulchery, CITH- W-9		
(+)		Triangular	2	CITH-W-26	d	VS
		Cordate	3	Nugget, CITH-W-85		
		Ovate	4	Cheinovo, Hamdan, CITH-W-2, CITH-W-6, CITH-W-5		
		Short	5	CITH-W-32, CITH-W-36		
		Trapezoid				
		Long	6	CITH-W-1,CITH-W-10,CITH-W-8		
		Trapezoid	7			
		Broad Elliptic	7	CITH-W-29, CITH-W-37, CITH- W-45		
		Elliptic	8	Franquette, CITH-W-7		
		Narrow Elliptic	9	CITH-W-42, CITH-W-70		
22. (+)	Nut: Shape in cross	Oblate	3	Franquette, CITH-W-1, CITH-W-4, CITH-W-8, CITH-W-10	d	VS
	section	Round	5	Sulaiman, Opex Caulchery, CITH- W-2, CITH-W-5, CITH-W-6		
		Elliptic	7	Cheinovo, Hamdan, CITH-W-3, CITH-W-7		
23.	Nut: Shape	Cuneate	1	Cheinovo, CITH-W-7		
(+)	of base	Rounded	3	Franquette	d	VS
	perpendicul ar to suture	Truncate	5	Sulaiman, CITH-W-1 CITH-W-2, CITH-W-9		
		emarginate	7	Nugget, CITH-W-12		
24. (+)	Nut: Shape of apex	Pointed	1	Cheinovo, CITH-W-2, CITH-W-3, CITH-W-7	d	VS
	perpendicular	Rounded	3	CITH-W-6, CITH-W-5, CITH-W-8		
	to suture	Truncate	5			
		emarginate	7			
25.	Nut:	Weak	3	Opex Caulchery, CITH-W-11		
(*) (+)	Prominence of apical tip	Medium	5	CITH-W-1,CITH-W-8,CITH-W-4, CITH-W-10	d	VS
$X^{+}I$		Strong	7	CITH-W-42, CITH-W-70		
26. (+)	Nut: Position of	on upper half	1	Hamdan, CITH-W-2, CITH-W-4, CITH-W-8		
、 /	pad on suture	on upper 2/3	3	Franquette, Sulaiman, Tutle, Nugget, CITH-W-1, CITH-W-7	d	VS

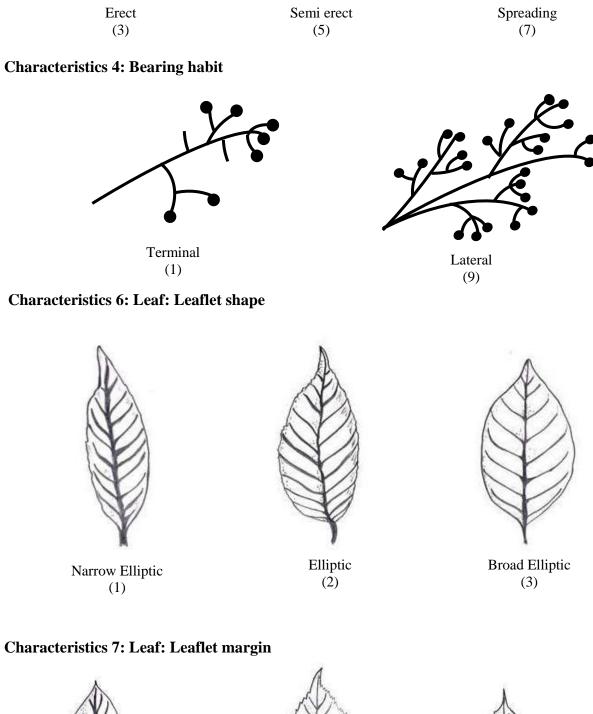
		on whole length	5	Cheinova, CITH-W-6,CITH-W-11		
27.	Nut	Small (<30)	3	Fanquette, Tutle		
27.	diameter	Medium	5	Sulaiman, Cheinova, Opex		
	(mm)	(30-40)		Caulchery, Hamdan, CITH-W-3, CITH-W-5	d	MG
		Large ( > 40)	7	CITH-W-1,CITH-W-2, CITH-W-8		
28.	Nut length (mm)	Small (< 30)	3	Tutle		
		Medium (30-40)	5	Opex Caulchery, Nugget, CITH-W- 3, CITH-W-11, CITH-W-12	d	MG
		Large (>40)	7	Hamdan, Cheinovo, Sulaiman, CITH-W-1, CITH-W-2, CITH-W-4		
29.	Nut weight (g)	Light (<15)	3	Opex Caulchery, Cheinovo, CITH- W-11	d	MG
		Medium (15-20)	5	Hamdan, Sulaiman, CITH-W-2		
20	Nur4	Heavy (>20)	7	CITH-W-1, CITH-W-5, CITH-W-8		
30.	Nut: prominence	Weak	1	Tutle, Hamdan, CITH-W-1, CITH- W-2	d	VS
(+)	of pad on suture	Medium	3	Franquette, Sulaiman CITH-W-11, CITH-W-13	u	v S
		Strong	5	Cheinova, Franquette, CITH-W-6		
31.	Shell surface	Smooth	3	Hamdan, CITH-W-1		
(*) (+)		Moderately Smooth	5	Nugget, Cheinovo, Opex Caulchery, Tutle, CITH-W-13	d	VS
		Rough	7	Sulaiman, CITH-W-2		
32.	Shell colour	Very light	1	CITH-W-46, CITH-W-73		110
(*)		Light	3	Hamdan, Nugget, CITH-W-1, CITH-W-2, CITH-W-4,CITH-W-5, CITH-W-8	d	VS
		Medium	5	Opex Caulchery, Cheinovo, CITH- W-7		
		Dark	7	CITH-W-74, CITH-W-75		
33.	Shell seal	Weak	3	Hamdan		
		Intermediate	5	Sulaiman, CITH-W-1, CITH-W-5, CITH-W-6, CITH-W-8		
		Strong	7	Opex Caulchery, Cheinovo, Tutle, CITH-W-10,	d	VS
24	Ch P	Very strong	9	Franquette, Nugget, CITH-W-3		
34. (*)	Shell strength	Weak Intermediate	3	Cheinovo Hamdan , Sulaiman, Nugget,	d	MG
(*)	suchgui	Strong	5 7	Hamdan , Sulaiman, Nugget, CITH-W-2 CITH-W-14, CITH-W-28, CITH-	u	
				W-42		
35.	Shell	Weak	3			
	integrity	Intermediate	5	Hamdan,		
		Strong	7	Open Caulchery, Tutle, Nugget, Cheinovo, Sulaiman, Franquette, CITH-W-2,CITH-W-3, CITH-W-4, CITH-W-5,CITH-W-6	d	VS

36.	Shell	Thin ( <1)	1	Hamdan, CITH-W-24, CITH-W-36		
50.	thickness	Medium	2	Nugget, Opex Caulchery, Nugget,		
	(mm)	(1-2)	-	Cheinovo, CITH-W-2, CITH-W-3		
		Thick (>2)	3	CITH-W-19	d	MG
37.	Kernel	Light (<6)	3	Opex Caulchery, Nugget		
	weight	Medium	5	Hamdan		
	(g)	6-10)				
		Heavy(>10)	7	CITH-W-1, CITH-W-6, CITH-W-7	d	MG
38.	Kernel	Low (<40)	3	CITH-W-55		
	percentage	Medium	5	Opex Caulchery, Nugget, ITH-W-2		
		(40-50)				
		High( 50-60)	7	Hamdan, Tutle, CITH-W-1,		
		Very high	9	CITH-W-58, CITH-W-38		
		(>60)				
39.	Kernel veins	Low (<30)	1	Tutle, Opex Caulchery, CITH-W-10		
(*)	(%)	Medium	2	CITH-W-5 CITH-W-6		
		(30-40)			d	MG
		High (> 40)	3	Nugget,CITH-W-52, CITH-W-54		
		Very high	4	Sulaiman,CITH-W-55, CITH-W-56		
		(>50)				
40.	Kernel	Thin	3	CITH-W-55,CITH-W-57		
	plumpness	Moderate	5	CITH-W-23, CITH-W-36	d	VG
		Plumpy	7	Tutle, Hamdan, Nugget, Opex		
				Caulchery Sulaiman, Cheinovo,		
44	Ease of	East	3	CITH-W-2,CITH-W-5		
41.	Lase of removal of	Easy	3	Tutle, Nugget, Cheinovo, Opex Caulchery, Sulaiman, Franquette,		
(*)	kernel			CITH-W-1,CITH-W-2,CITH-W-6,	d	MG
	halves			CITH-W-10	u	MO
	nurves	Moderate	5	CITH-W-5,CITH-W-11,CITH-W-3		
		Difficult	7	CITH-W-36, CITH-W-66		
42.	Kernel	Extra light	1	Hamdan, CITH-W-1, CITH-W-5		
	colour	Light	2	Franquette, Nugget, Opex	d	VS
				Caulchery, Cheinovo, CITH-W-2		
		Amber	4	CITH-W-7, CITH-W-36, CITH-W-		
				82,		
		Dark amber	7	Tutle		

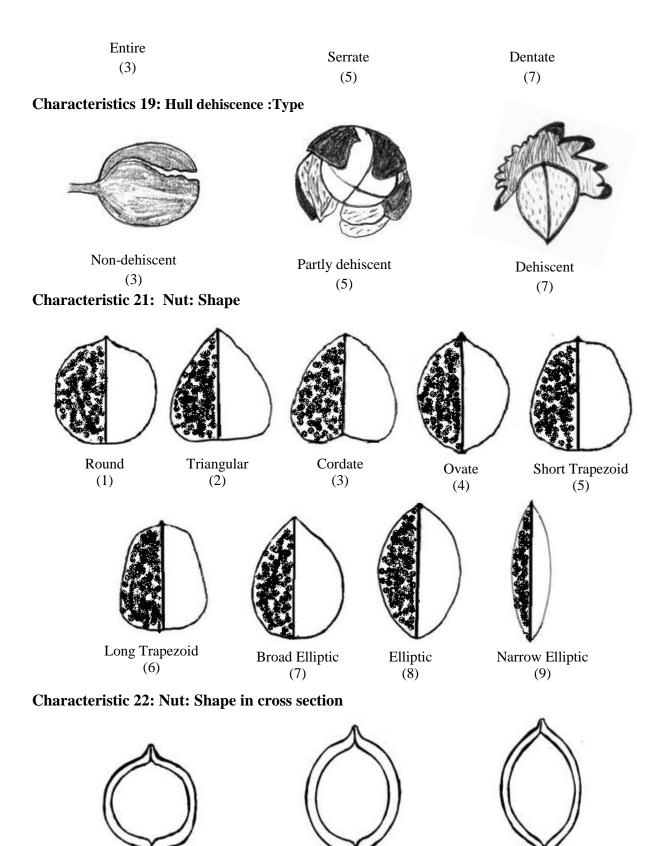
VIII. Explanation for the Table of characteristics

**Characteristics 2: Tree growth habit** 

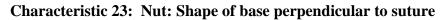


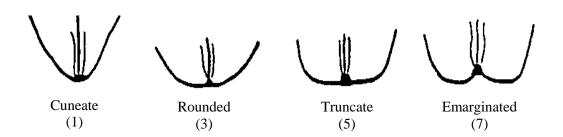




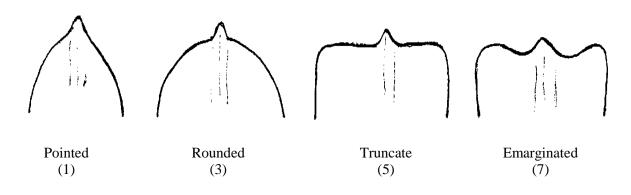


Oblate	Round	Elliptic
(3)	(5)	Emptic
		(7)

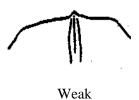




Characteristic 24: Nut: Shape of apex perpendicular to suture



Characteristic 25: Nut: Prominence of apical tip



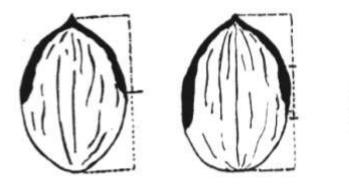
(3)



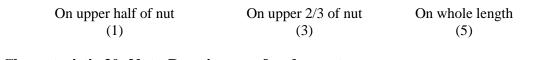
Medium (5)

Strong (7)

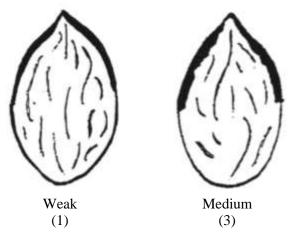
#### Characteristic 26: Nut: Position of pad on suture







## Characteristic 30: Nut: Prominence of pad on suture





Strong (5)

Characteristic 31: Nut: Shell surface



Smooth (3)



Moderately smooth (5)



Rough (7)

#### Working Group details:

The Test Guidelines were developed at Central Institute of Temperate Horticulture, Srinagar, J&K. Under the supervision of Prof. Nazeer Ahmed, Director as PI and Assisted by Dr. S. R. Singh, Sr. Scientist and Research Associates Dr. Megna Rashid, Dr. Hidayat-ullah-Mir, Mr. P. A. Shagoo and Dr. Tejbir Singh, Registrar, PPV&FRA New Delhi. The suggestions and technical inputs were provided by the following task force (4/2012) constituted by the PPV&FR Authority in development and finalization of this DUS test guidelines.

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Nodal DUS Test Centre	Other DUS Test Centre
Central Institute of Temperate Horticulture, Rangreth, Srinagar (J&K)	