GUIDELINES
FOR THE CONDUCT OF TEST FOR
DISTINCTIVENESS, UNIFORMITY AND STABILITY

On

KALMEGH

(*Andrographis paniculata)* (Burm.f.) Wall. ex Nees)

Protection of Plant Varieties and Farmers' Rights Authority

(PPV & FRA)

Government of India

Contents

DRAFT GUIDELINES FOR CONDUCTING DUS TEST ON KALMEGH

(*Andrographis paniculata*) (Burm.f.) Wall. ex Nees)

I. Subject

These test guidelines shall apply to all varieties, parental lines and hybrids of Kalmegh (*Andrographis paniculata*) (Burm.f.) Wall. ex Nees

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 is required for testing the 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.
2. The minimum quantity of the seeds for each variety to be provided by the applicant shall be divided equally in packets as per the following:
   i) New variety: 30 gm in 10 packets
   ii) Varieties of Common knowledge and farmers' varieties:15 gm in 5 packets
3. The seeds shall have the following standards
   i) Germination: at least 95 % at 25º C,
   ii) Purity: 98% physical purity, highest genetic purity & uniformity,
   iii) Moisture content shall not exceed 8-9%
4. The seeds shall meet highest sanitary and phyto-sanitary standards. In addition, the applicant shall also submit along with the seed, a certified data on germination test made not more than one month prior to the date of submission. The seed material submitted shall not have undergone any chemical and bio-physical treatment unless the PPV&FR authority allows or requests for such treatment. If it has been treated, full details of the treatment shall be given.

III. Conduct of tests

1. The minimum duration of the DUS tests shall normally be at least two independent similar growing seasons for all new varieties and for one growing season for varieties of common knowledge and farmers' varieties.
2. The test shall normally be conducted at two test locations. If any essential characteristic(s) of the candidate variety are not expressed for visual observation at these locations, the variety may be considered for further examination at another appropriate test site and under special test protocol on expressed request of the applicant.
3. The field tests shall be carried out under conditions ensuring normal growth and expression of characters included in the test. The size of the plots shall be such that plants or parts of plants could be removed for measurement and counting without prejudice to observations on the standing plants until the end of the growing period. Each test shall include about 150 plants in the plot size (50 in each replication) 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 replications shall be sharing similar environmental conditions of the test location.

4. Test plot design

Plot size	:	4.50 X 3.00 m
Number of rows	:	5
Row length	:	4.50 m
Row to row distance	:	60 cm
Plant to plant distance	:	45 cm
Expected plants / replication	:	50

Number of replications : 3

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

IV. Methods and observations

1. The characteristics described in the Table of characteristics shall be used for the testing of varieties, parental lines and hybrids of Kalmegh (*Andrographis paniculata*) for the conduct of DUS.
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 3 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 1% with an acceptance probability of at least 95% should be applied. For the assessment of Uniformity of characteristics on a plot as a whole (visual assessment by observations of a number of individual rows, plants or parts of plants), the number of aberrant or odd type plants or parts of plant shall not exceed 1 in 150.
4. For the assessment of colour characteristics, the latest Royal Horticultural Society (RHS) Colour Chart shall be used.
5. All observations on leaf shall be made from leaves of nodes between the 5^{th} and 9^{th} node from the bottom on the main stem. Records on quantitative characters like leaf length and breadth shall be taken from 60 leaves harvested from 30 plants out of 3 replications.
6. Measurements shall be made in metric units.

V. Grouping of varieties

1. The candidate varieties for the 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 shall be used for grouping varieties

a) Leaf: Lamina: Breadth (Characteristic no.4)

b) Stem: Branching pattern (Characteristic no.8)

c) Anthesis: Pattern (Characteristic no.9)

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 their 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 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. 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.

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

*** Decimal code for the growth stage**

Growth Stage	**Decimal code**
Sowing	00
Germination	04
Transplanting	25
Branching initiation	44
Reproductive branching Initiation	53
Canopy initiation	56
Flowering/Anthesis initiation	63
Adoption of final branching pattern	69
Peak Flowering	88
Peak Fruiting	97
Canopy final shape	100
Harvesting	100

***** Total growth period of about 160 days (including 40 days in nursery growth) was converted to decimal scale

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 observation (code)	Type of assessment
1	2	3	4	5	6	7
1. (*)	Leaf: Colour	Light green (RHS colour chart Yellow Green Group- 146 A, B)	3	DMAPR AP 19	53	VG
		Green (RHS colour chart Green Group- 137 A, B)	5	DMAPR AP 61		
		Dark green (RHS colour chart Green Group- 137N A, B)	7	DMAPR AP3		
2. (*) (+)	Leaf: lamina shape	Lanceolate	3	DMAPR AP 3, DMAPR AP 6, DMAPR AP 68	56	VS
		Elliptical	5	DMAPR AP 48		
		Ovate /Ovate-elliptical / Ovate - lanceolate	7	DMAPR AP 1, DMAPR AP 19, DMAPR AP24		
3. (*) (+)	Leaf : Lamina: Length	Short (Length <9.00 cm)	3	DMAPR AP 6, DMAPR AP 27	56	MS
		Long (Length >9.0 cm)	7	DMAPR AP 1, DMAPR AP 3		
4. (*) (+)	Leaf: Lamina: Breadth	Narrow (Breadth <1.5 cm)	3	DMAPR AP3, DMAPR AP 6	56	MS
		Medium (Breadth 1.5 to 2.5 cm)	5	DMAPR AP 2, DMAPR AP 27		
		Broad (Breadth >		DMAPR AP 1,		

		2.5 cm)	7	DMAPR AP 24		
5. (*) (+)	Stem :Shoot apex	Tender leaves grouped at apex (rosette)	1	DMAPR AP 19	56	VS
		Tender leaves not grouped at apex	9	DMAPR AP 1 DMAPR AP 2		
6. (+)	Leaf: Lamina curvature	Inwardly curved (incurved)	3	DMAPR AP15	56	VS
		Outwardly curved (reflexed)	7	DMAPR AP16		
7. (*)	Leaf: Lamina surface	Smooth	3	DMAPR AP37	56	VS
		Wrinkled	7	DMAPR AP 2		
8. (*) (+)	Stem: Branching pattern	Erect	3	DMAPR AP19	56	VS
		Spreading	7	DMAPR AP 22 DMAPR AP 69		
9. (*)	Anthesis Pattern	Early [Anthesis initiation <70Days after transplanting]	3	DMAPR AP 37	69	VG/MG
		Medium (Anthesis initiation 70-100 Days after transplanting)	5	DMAPR AP 6		
		Late (Anthesis initiation > 100 Days after transplanting)	7	DMAPR AP 61		
10. (+)	Inflorescence (Panicle): Rachis type (Arrangement of flower buds on rachis)	Flower buds closely arranged (Dense rachis)	3	DMAPR AP 6	88	VG
		Flower buds distantly arranged (Loose rachis)	5	DMAPR AP 37		
11. (+)	Plant: Main Axis Growth habit	Erect	3	DMAPRAP2, DMAPR AP19	88	VS

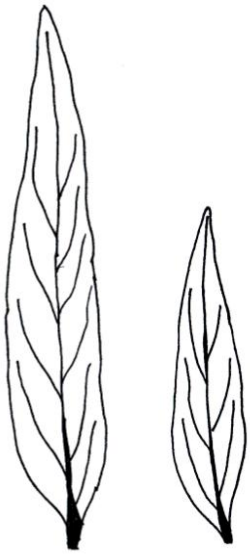
		Prostrate	5	DMAPR AP 21 DMAPR AP3		
12. (*) (+)	Stem internode: Length	Short (Internode length <3 cm)	3	DMAPR AP 6	88	MSS
		Long (Internode length > 3 cm)	7	DMAPR AP 22		
13. (*) (+)	Plant canopy: Shape	Columnar	3	DMAPR AP19	88	VS
		Bushy/Globular	5	DMAPR AP 4, DMAPR AP 61		
		Pyramidal	7	DMAPR 10 DMAPR AP57 DMAPR AP46		
14. (+)	Plant: Height	Short (<50 cm)	3	DMAPR AP 56	97	MS
		Medium (50- 70 cm)	5	DMAPR AP 2 DMAPR AP 19		
		Tall (> 70 cm)	7	AK 1 DMAPR AP 51		
15. (+)	Leaf: Andrographolide ($C_{20}H_{30}O_5$) content % on dry weight basis	Low	3	DMAPR AP 45	100	MG
		Medium	5	DMAPR AP 68		
		High	7	DMAPRAP19		

***Anthesis= opening of flower in the season**

VIII. Explanation on the Table of Characteristics

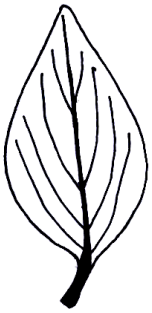
Characteristic 2. Leaf: Lamina shape

Leaf lamina shape shall be made from leaves of nodes between the 5th and 9th node from the bottom on the main stem.



Lanceolate (3)

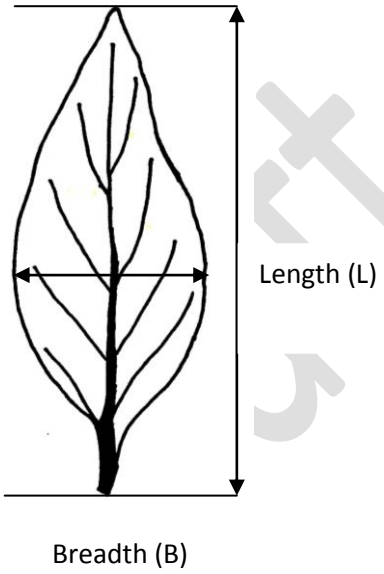
Elliptical (5)



Ovate/ Ovate-elliptical /Ovate –lanceolate (7)

Characteristic 3& 4. Leaf: Lamina length and breadth

Records on quantitative characters like leaf lamina length (L) and breadth (B) shall be taken from a sample size of 60 leaves. Observations shall be made on the leaves from the 5^{th} to 9^{th} nodes from the bottom on the main stem shall be selected. Breadth (B) shall be taken from the broadest lamina area.



Characteristic 5. Stem : Shoot Apex

Tender leaves grouped at shoot apex: rosette (1)

Tender leaves not grouped at shoot apex(9)

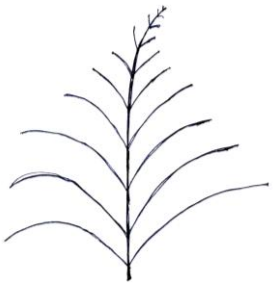
Characteristic 6. Leaf: Lamina curvature

Inwardly curved : incurved(3)

Outwardly curved : reflexed(7)

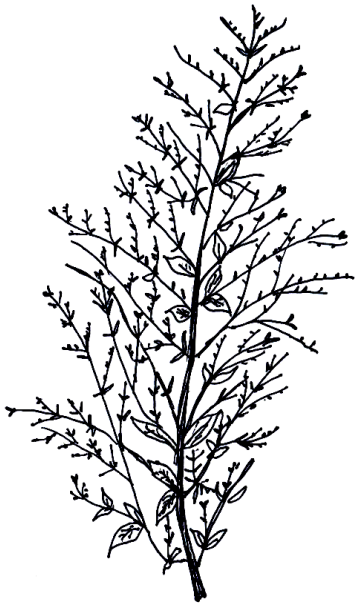
Characteristic 8. Stem : Branching pattern

Erect(3)

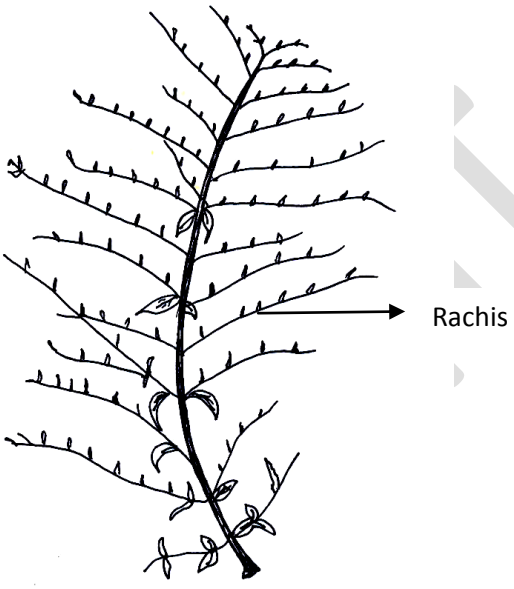


Spreading (7)

Characteristic 10. Inflorescence (Panicle): Rachis type



Flower buds closely arranged on the rachis : Dense rachis (3)



Flower buds distantly arranged on the rachis: Loose rachis (5)

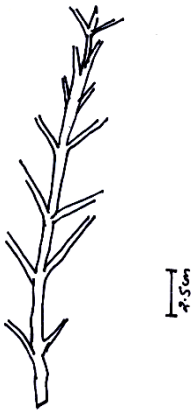
Characteristic 11. Plant: Main Axis Growth habit:

Erect(3)

Prostrate(5)

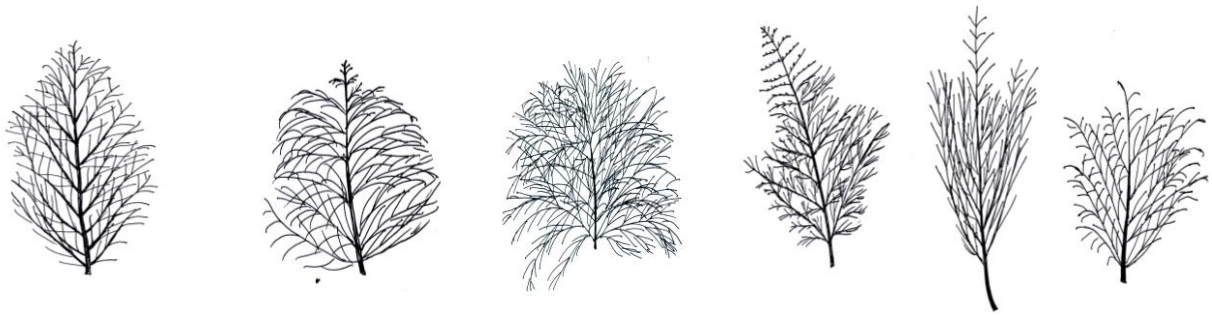
Characteristic 12. Stem internode: Length

Short < 3 cm(3)



Long > 3 cm(7)

Characteristic 13. Plant Canopy : Shape



Columnar (3)) Bushy/Globular (5) Pyramidal (7)

Characteristic 14. Plant: Height

Plant height shall be recorded by taking measurement from base of the plant to top of the canopy as shown below.

Plant Height

Characteristic 15. Andrographolide Content

Andrographolide ($C_{20}H_{30}O_5$) estimation shall be done by HPLC method (Gajbhiye and Khristi, 2010) as follows:

Dried leaf material is ground in cyclone mill and powdered material is used for the andrographolide estimation. One gram sample powder is extracted with mixture of dichloromethane (CH_2Cl_2) and methanol (≥99.9% CH_3OH) in the ratio of 1:1 by cold maceration. The extracts are filtered through Whatman filter paper Grade 1 and evaporated on rotary evaporator. The dark green residue is washed with toluene. After complete removal of toluene (C_7H_8) samples are re-dissolved in HPLC grade methanol for HPLC analysis. The HPLC system (Shimadzu, Japan) consisting of LC-10AD pumps and SPD-10A UV-VIS detector at wavelength 229 nm along with Aimil chromatographic data station is used for andrographolide estimation with RP-18 column (250 mm × 4.6 mm, 5 µm, Merck). The analysis is carried out in mobile phase 65:35 of methanol and water at a flow rate 1 ml min^{-1}. Calibration curve for andrographolide is constructed by standard andrographolide (Sigma Aldrich, USA). The calibration curve is obtained by loading different concentrations of andrographolide against peak area. The calibration equation obtained is used for sample analysis.

Literature:

1. Gajbhiye, N.A. and S. Khristi. 2010. Distribution pattern of andrographolide and total lactones in different parts of Kalmegh plant. *Indian Journal of Horticulture*, 67(4): 591-593.
2. Hickey, Michael and Clive King 2000. The Cambridge illustrated glossary of botanical terms. Cambridge University Press. pp 1-208.

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