

Draft
Guidelines
For the Conduct of Test for
Distinctiveness, Uniformity & Stability
on

Lemongrass
[*Cymbopogon flexuosus* L.]



Protection of Plant Varieties and Farmers' Rights
Authority
(A Statutory Body created by an Act of Parliament)
Government of India, New Delhi

CONTENTS

S. No	Particulars
I.	Subject
II.	Material required
III.	Conduct of tests
IV.	Methods and observations
V.	Grouping characteristics
VI.	Characteristics and symbols
VII.	Table of characteristics
VIII.	Explanations for the Table of characteristics
IX.	Working Group details
X.	Name of DUS Test Centre

I. Subject

These test guidelines shall apply to all vegetatively propagated varieties and parental lines of Lemongrass (*Cymbopogon flexuosus* L.).

II. 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 Variety and Farmers' Rights (PPV & FR) Act, 2001. Applicants submitting such planting 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 planting material shall be supplied from pure 8– 10 months old plants, visibly healthy and not lacking in vigour or affected by any pest or disease. It should be obtained from *in- vivo* propagation and shall possess highest genetic purity, uniformity, sanitary and phyto-sanitary standards.
3. The planting material shall not have been subjected to any chemical or bio-physical treatment unless the PPV&FR Authority allows or request such treatment. If it has been treated full details of the treatment must be given. The sets with healthy slips shall be carefully packed without damaging lower rooted portions.
4. The minimum quantity of planting material to be provided by the applicant shall be **150** fresh viable slips per centre.

III. Conduct of tests

1. The minimum duration of DUS tests shall be of two independent similar growing seasons. The test shall normally be conducted at least at two test locations for new category and one testing season for extant/ farmer's category.
2. If any essential characteristics of the candidate variety are not expressed observation/ measurement at these locations, the variety shall be considered for further examination at another appropriate test site or under special test protocol on request expressed by 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 measurement and observation without prejudicing the other observations on the standing plants till the end of the growing period. Each test shall include at least a **minimum 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. The test plot design shall be as follows-

Number of rows	: 4
Row length	: 6 m
Row to row distance	: 60 cm
Plant to plant distance	: 60 cm
Number of replications	: 3

5. Observations shall not be recorded on plants in border rows. Any kind of Plant Promoting Growth Hormones should not be used throughout the growth period (vegetative/ flowering period).

6. Additional tests for special purpose shall be established by the PPV & FR Authority.

IV. Methods and observations

1. The characteristics described in the Table of characteristics (refer section VII) shall be used for the DUS testing of varieties.
2. For the assessment of Distinctiveness and Stability, observations shall be made on at least **10 slips/ replication**, unless otherwise indicated (In Lemongrass, the above ground part known as ‘slip’ is a cluster of culms derived from a single plant of Lemongrass, which is analogous to a single plant.
3. For the assessment of Uniformity of single lines a population standard of 1% with an acceptance probability of 95% shall be applied. In the case of a sample 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 slips shall be made at mid height of the fully developed crop.
6. All observations on nodes and internodes shall be made at mid height of the fully developed crop.
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.

Methodology adopted for recording of observations for Qualitative and Quantitative characters.

S. No	Character	Methodology
1	Vegetative plant height	Vegetative plant height was measured in centimetre from ground level to the tip of the highest leaf of the plant on maturity by a meter scale.
2	Plant habit	The growth habit of the plant was visually assessed from the altitude of the leaves and tillers. The angle formed by the outer leaves and tillers with an imaginary vertical axis was used.
3	Leaf length (cm)	Leaf length was measured in centimetre from just above the basal sheath to the tip of the lamina.
4	Leaf breadth (cm)	Leaf breadth was measured in centimetre at the middle portion of the lamina.
5	Leaf drooping behaviour	The drooping behaviour of the crop was determined by observing the bending of leaf downwards.
6	Leaf midrib colour	The colour of the leaf midrib was observed and matched with the RHS colour chart.
7	Leaf sheath colour	Colour of the leaf sheath was observed and recorded at matured stage as- light green, dark green and normal green and matched with RHS colour chart.
8	Length of basal sheath	This measure was taken from the base of the plant to the beginning of the lamina.
9	Colour of basal sheath	Colour of the basal sheath was observed and recorded with naked eye at matured stage as- green and red and matched with RHS colour chart.
10	Tillers/clump	Number of tillers was counted by the total number of tillers per clump at matured stage.
11	Stem colour	The colour of the stem was observed and matched with the RHS colour chart.
12	Internode colour	The colour of the internode was observed and matched with the RHS colour chart.
13	Auricle colour	The colour of the auricle was observed and matched with the RHS colour chart.

14	Inflorescence colour	The colour of the inflorescence was observed and matched with the RHS colour chart.
15	Length of spike	This measure was taken from a node which emerges from the panicle up to tip of the inflorescence and was measured in centimetres scale.
16	Inflorescence type	The type of inflorescence was determined by observing the arrangement of the cluster of flowers on floral axis.
17	Colour of exposed stem	This character was visualized and recorded at maturity as- deep reddish brown and light yellowish green and matched with RHS colour chart.
18	Number of nodes	The number of nodes was counted from the ground level to the base of the inflorescence from where spikes emerged.
19	Girth of node	Girth of node was taken with the help of a thread and then the thread was measured with the help of scale.
20	Essential oil quantity	Essential oil was extracted using Clevenger apparatus for 3 h. The quality of the essential oil was analysed using Gas Chromatography FID and GC-MS.

V. Grouping Characteristics

The following characteristics shall be used for grouping Lemongrass varieties.

- a) Plant habit (characteristics 2)
- b) Leaf drooping behaviour (characteristics 5)
- c) Inflorescence type (characteristics 16)

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 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 in all the varieties and shall always be included in the description of the variety. In

exceptional cases, whereas the state of expression of any of these characters is not recorded due to environmental vagaries, adequate explanation shall be provided.

(+) is mentioned wherever sketches/ photographs are given. The explanation for the characteristics is provided in (section VIII) for clarity.

4. Characteristics containing the following key in the first column of the Table of characteristics shall be examined as indicated below:

QL: Qualitative characteristic

QN: Quantitative characteristic

QL: Qualitative characteristics are those that are expressed in discontinuous states.

These states are self-explanatory and independently meaningful. All states are necessary to describe the full range of the characteristic and every form of expression can be described by a single state. As a rule, the characteristics are not influenced by environment.

QN: Quantitative characteristics are those where the expression covers the full range of variation from one extreme to the other. The expression can be recorded on a one-dimensional, continuous or discrete, scale. The range of expression is divided into a number of states for the purpose of description.

5. 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
60	End of fully growth stage
90	Maturity stage (Flowering time)
100	Harvest stage

6. Type of assessment of characteristics indicated in column six of Table of characteristics VII 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	Notes	Example Variety	IC No	Stage of observation	Types of Assessment
1	2	3	4	5		6	7
1. (+) QN	Vegetative plant height (cm)	Short (<75 cm)	3	RRLJM-404, CKP 25	IC-0626693	60	MS
		Medium (75-150 cm)	5	RRLJM-215 Krishna	IC-0626580		
		Tall (>150-200 cm)	7	RRLJM-228	IC-0626592		
		Very tall (>200 cm)	9	RRLJM-285	IC-0626643		
2. (+) QL	Plant habit	Erect	3	RRLJM-260	IC-0626621	90	VG
		Semi erect	5	RRLJM-304	IC-0626652		
		Drooping	7	RRLJM-284	IC-0626642		
3. (+) QN	Leaf length (cm)	Short (<50 cm)	3	RRLJM-401	IC-0626661	90	MS
		Medium (50-100 cm)	5	RRLJM-272	IC-0626631		
		Long (>100 cm)	7	RRLJM-524	IC-0626716		
4. (+) QN	Leaf breadth (cm)	Narrow (<1 cm)	3	RRLJM-270 CKP 25	IC-0626629	90	MS
		Medium (1-2 cm)	5	RRLJM-256	IC-0626617		
		Broad (>2 cm)	7	RRLJM-216	IC-0626581		
5. (+) QL	Leaf drooping behaviour	Erect	3	RRLJM-620	IC-0626765	90	VG
		Semi erect	5	RRLJM-605	IC-0626750		
		Drooping	7	RRLJM-679	IC-0626824		
6. (+) QL	Leaf midrib colour	Strong Yellow Green (RHS- 143 C)	1	RRLJM- 523	IC-0626715	90	VS
		Light Bluish Green (RHS-133 C)	3	RRLJM- 537	IC-0626729		
7. (+) QL	Leaf sheath colour	Strong Yellow Green (RHS- 143 B)	3	RRLJM- 3H8	IC- RRLJ3H8	90	VS
		Strong Yellow Green (RHS 144 C)	5	RRLJM- 438	IC- RRLJ5H3		
		Deep Pink (RHS 180 D)	7	RRLJM- 285	IC-0626643		
8. (+) QN	Length of basal sheath (cm)	Short (<10 cm)	3	RRLJM 543	IC-0626735	90	MS
		Medium (10-30 cm)	5	RRLJM 221	IC-0626586		
		Long (>30 cm)	7	RRLJM 250	IC-0626612		
9. (+) QL	Colour of basal sheath	Strong Yellow Green (RHS- 143 B)	3	RRLJM- 259	IC-0626620	90	VS
		Greyish Brown (RHS- 166 A)	7	RRLJM- 284	IC-0626642		
10. (+) QN	Tillers per clump	Sparse (<40)	3	RRLJM-444	IC-0626696	90	MS
		Medium (40-80)	5	RRLJM-522	IC-0626714		
		Profuse (>80)	7	RRLJM-412	IC-0626670		

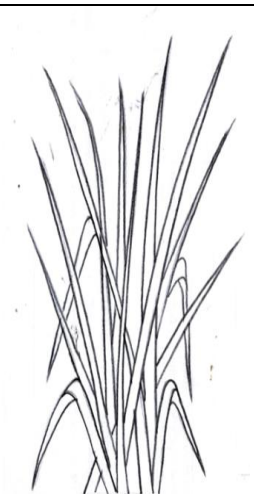
11. (+) QL	Stem colour	Light Greenish Yellow (RHS 4 B)	3	RRLJM- 259	IC-0626620	90	VS
		Vivid Red (RHS 46 B)	5	RRLJM- 273	IC-0626632		
		Light Purplish Pink (RHS 55 C)	7	RRLJM- 248	IC-0626610		
12. (+) QL	Internode colour	Light Greenish Yellow (RHS 6 D)	3	RRLJM- 201	IC-0626568	90	VS
		Vivid Red (RHS 45 C)	5	RRLJM- 248	IC-0626610		
		Greyish Reddish Brown (RHS 200 B)	7	RRLJM- 215	IC-0626580		
13. (+) QL	Auricle colour	Strong Yellow Green (RHS 143 B)	3	RRLJM- 259	IC-0626620	90	VS
		Vivid Red (RHS 46 C)	5	RRLJM- 720	IC-0626852		
		Deep Purple (RHS 83 B)	7	RRLJM- 829	IC-0626874		
14. (+) QL	Inflorescence colour	Strong Yellow green (RHS- N144 C)	5	RRLJM-634	IC-0626779	90	VG
		Greyish Red (RHS- 182 B)	7	RRLJM-659	IC-0626804		
15. (+) QN	Length of spike (cm)	Short (<50 cm)	3	RRLJM 440	IC-0626693	90	MS
		Medium (50-100 cm)	5	RRLJM 230	IC-0626594		
		Long (>100 cm)	7	RRLJM 534	IC-0626735		
16. (+) QL	Inflorescence type	Loose	3	RRLJM-102	IC-0626990	100	VG
		Semi-compact	5	RRLJM-712	IC-0626844		
		Compact	7	RRLJM-854	IC-0626888		
17. (+) QL	Colour of exposed stem	Light Greenish Yellow (RHS- 8 C)	5	RRLJM-655	IC-0626800	100	VS
		Moderate Red (RHS-182 A)	7	RRLJM-5H3	IC-RLJ5H3		
18. QN	Number of nodes	Low (<4)	3	RRLJM-543	IC-0626735	100	MS
		Medium (4-6)	5	RRLJM-601	IC-0626754		
		High (>6)	7	RRLJM-420	IC-0626677		
19. (+) QN	Girth of node	Thin (<1 cm)	3	RRLJM-434	IC-0626688	100	MS
		Medium (1-2 cm)	5	RRLJM-285	IC-0626643		
		Thick (>2 cm)	7	RRLJM-420	IC-0626677		
20. QN	Essential oil percentage	Low (<0.5%)	3	RRLJM-546	IC-0626737	100	MG
		Medium (0.5-0.8%)	5	Krishna			
		High (>0.8%)	7	Jor Lab-L8 CKP 25 CIM-Shikhar			
21.	Major compound in the essential oil						
21.	Citral	Low citral	3	RRLJM-201	IC-0626568	100	MG

(a). QL		(≤45%)					
		Moderate citral (>45-65%)	5	BLI-ARUN	-		
		High citral (>65%)	7	RRLJM-223 CIM-Shikhar JOR LAB L-8	-		
21. (b). QL	Methyl eugenol	Low methyl eugenol (≤50%)	3	RRLJM-602	IC-0626747	100	MG
		High methyl eugenol (>50%)	7	RRLJM-634	JOR LAB L-9 INGR18037		
21. (c). QL	Elemicin	Low elemicin (≤50%)	3	RRLJM-524	IC-0626716	100	MG
		High elemicin (>50%)	7	RRLJM 546	JOR LAB L-10 INGR18039		

VII. Explanation for the Table of characteristics

Characteristics 1. Vegetative plant height:

	
Short (<75 cm) (3)	
	
Medium (75-150 cm) (5)	
	
Tall (>150-200 cm) (7)	

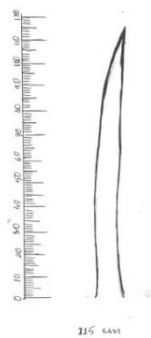


Very tall (>200 cm)
(9)

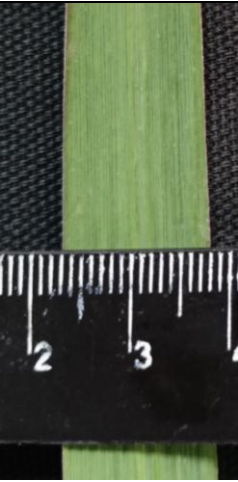
Characteristics 2. Plant habit:

	
Erect (3)	
	
Semi erect (5)	
	
Drooping (7)	

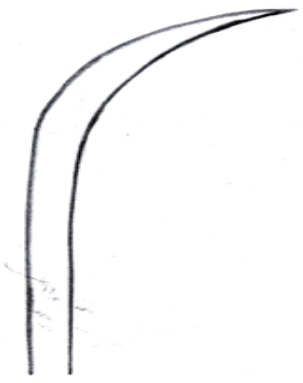
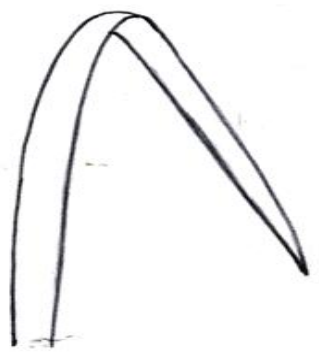
Characteristics 3. Leaf Length (cm):

 A drawing of a short, narrow leaf. To its left is a vertical ruler with markings from 0 to 30 cm. The leaf is approximately 4 cm long. Below the drawing, the text "4 cm" is written.	 A photograph of a short, narrow green leaf. To its left is a yellow ruler with markings from 0 to 10 cm. The leaf is approximately 4 cm long.
Short (<50 cm) (3)	
 A drawing of a medium-length, narrow leaf. To its left is a vertical ruler with markings from 0 to 120 cm. The leaf is approximately 90 cm long. Below the drawing, the text "90 cm" is written.	 A photograph of a medium-length green leaf. To its left is a yellow ruler with markings from 0 to 10 cm. The leaf is approximately 90 cm long.
Medium (50-100 cm) (5)	
 A drawing of a long, narrow leaf. To its left is a vertical ruler with markings from 0 to 180 cm. The leaf is approximately 115 cm long. Below the drawing, the text "115 cm" is written.	 A photograph of a long green leaf. To its left is a yellow ruler with markings from 0 to 10 cm. The leaf is approximately 115 cm long.
Long (>100 cm) (7)	

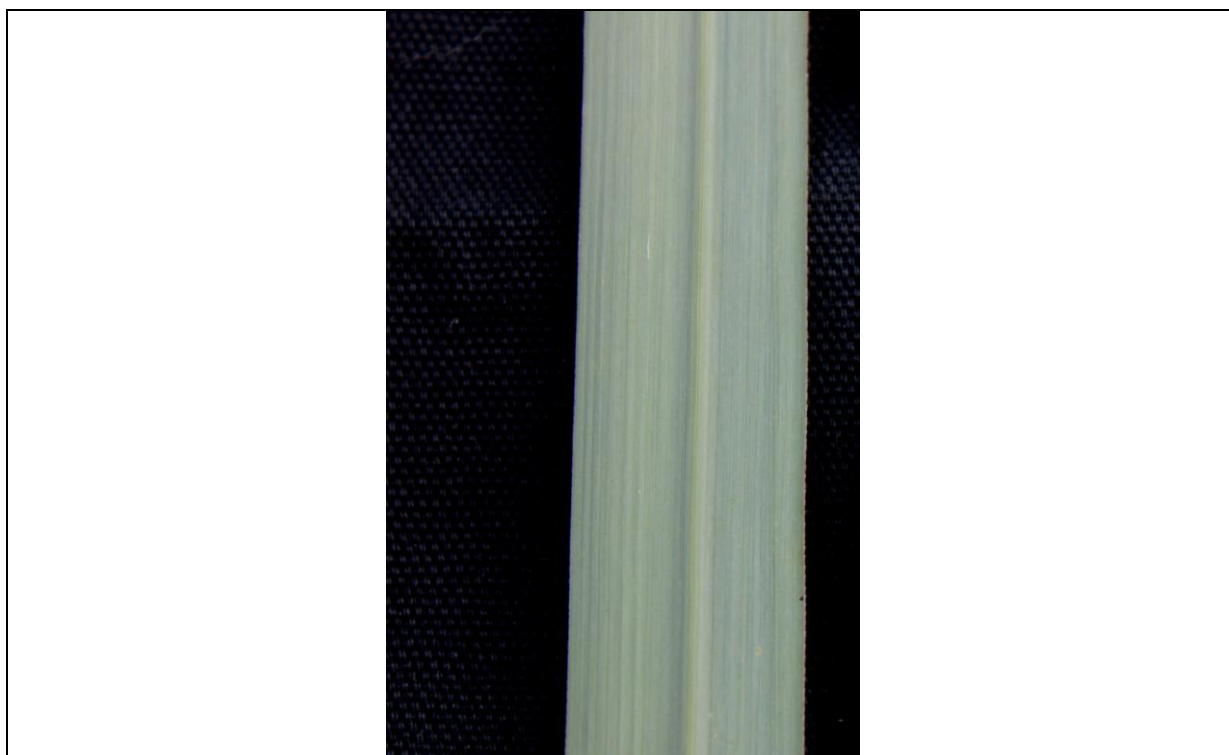
Characteristics 4. Leaf Breadth (cm):

	
Narrow (<1 cm) (3)	
	
Medium (1-2 cm) (5)	
	
Broad (>2 cm) (7)	

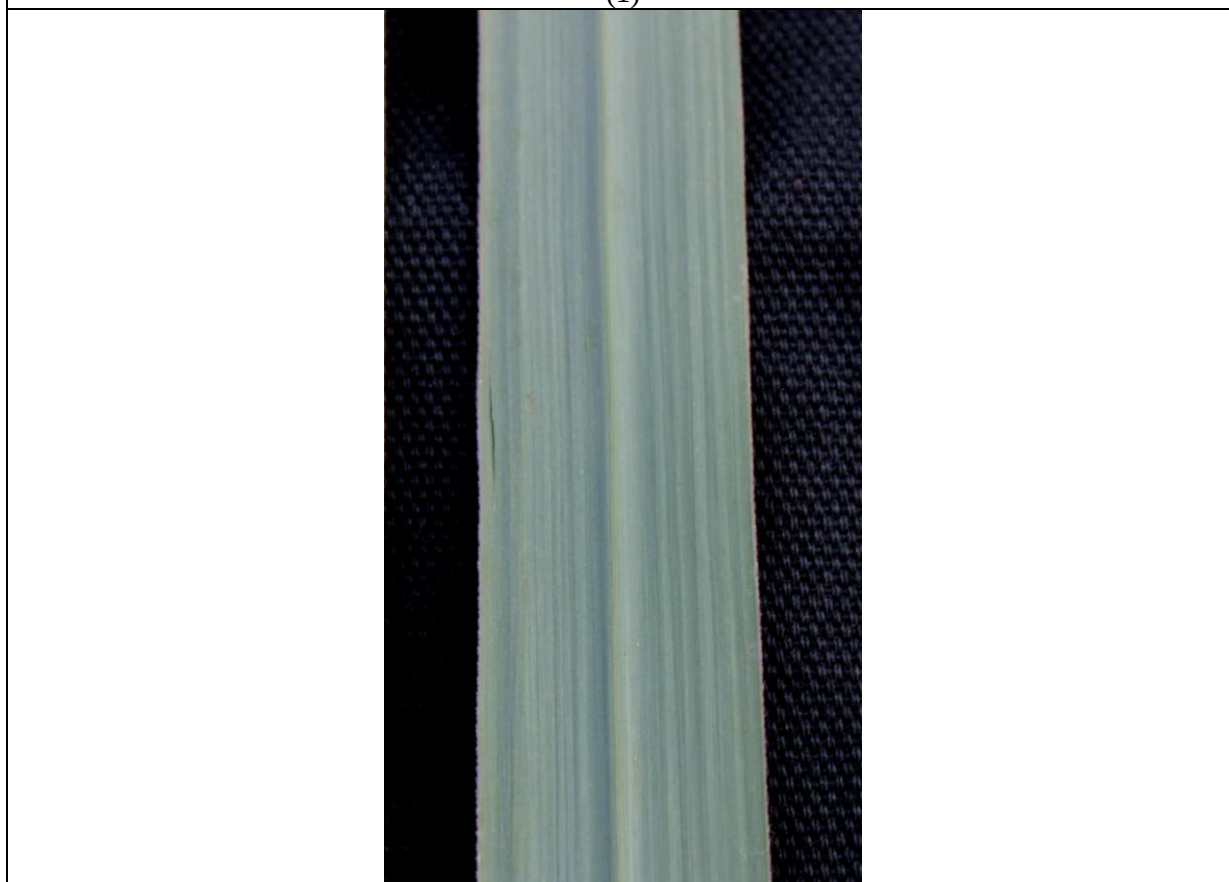
Characteristics 5. Leaf drooping behaviour:

	
Erect (3)	
	
Semi erect (5)	
	
Drooping (7)	

Characteristics 6. Leaf midrib colour:



Strong Yellow Green (RHS- 143 C)
(1)



Light Bluish Green (RHS-133 C)
(3)

Characteristics 7. Leaf sheath colour:



Strong Yellow Green
(RHS- 143 B)
(3)

Strong Yellow Green
(RHS 144 C)
(5)

Deep Pink (RHS 180 D)
(7)

Characteristics 8: Length of basal sheath (cm):



Short (<10 cm)
(3)



Medium (10-30 cm)
(5)

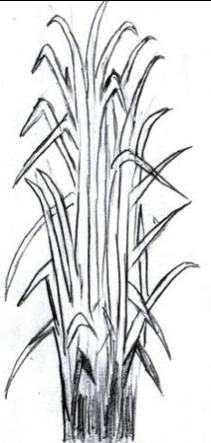


Long (>30 cm)
(7)

Characteristics 9. Colour of basal sheath:



Characteristics 10. Tillers per clump:

	
Sparse (<40) (3)	
	
Medium (40-80) (5)	
	
Profuse (>80) (7)	

Characteristics 11. Stem colour:



Characteristics 12. Internode colour:

		
Light Greenish Yellow (RHS 6 D) (3)	Vivid Red (RHS 45 C) (5)	Greyish Reddish Brown (RHS 200 B) (7)

Characteristics 13. Auricle colour:

		
RHS 143 B	RHS 46 C	RHS 83 B
Strong Yellow Green (RHS 143 B) (3)	Vivid Red (RHS 46 C) (5)	Deep Purple (RHS 83 B) (7)

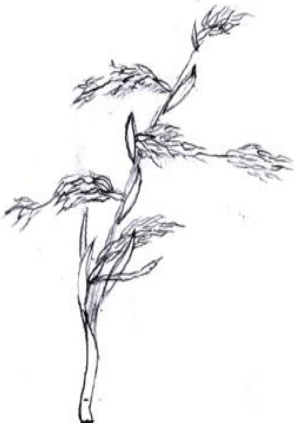
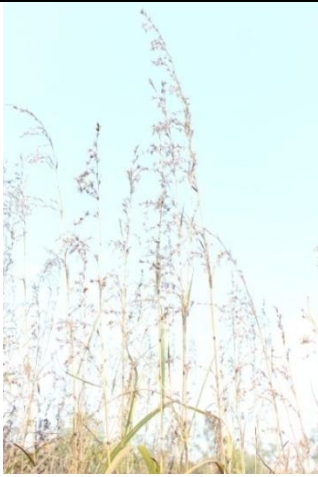
Characteristics 14. Inflorescence colour:

	
RHS N144 C	RHS 182 B
Strong Yellow green (RHS- N144 C) (5)	Greyish Red (RHS-182 B) (7)

Characteristics 15: Length of spike (cm):



Characteristics 16. Inflorescence type:

	
Loose (3)	
	
Semi compact (5)	
	
Compact (7)	

Characteristics 17. Colour of exposed stem:



Characteristics 19. Girth of nodes (cm):



20. Essential oil quality and quantity: Essential oil was extracted using Clevenger apparatus for minimum of 3 h. The quality of the essential oil was analysed using Gas Chromatography and GC-MS.

The essential oil content in percentage (w/w) was estimated on fresh weight basis. The essential oil concentration (%) was calculated by the following formula.

$$\text{Essential oil concentration (\%)} = \frac{\text{Amount of essential oil recovered (g)}}{\text{Amount of crop biomass distilled (g)}} \times 100$$

The essential oil extracted from the germplasm were categorized into three broad groups-

Low (0-0.4%): 0.4% (RRLJM 546)

Medium (0.5-0.8%): 0.80% (RRLJM 7); 0.81% (RRLJM 634)

High (>0.8): 1.1% (RRLJM 223); 1.2% (RRLJM 25)

Gas Chromatograph (GC-FID and GC/MS) conditions used for essential oil analysis

	GC-FID	GC-MS
Instrument	Thermo Scientific TRACE 1110 or equivalent model	TRACE Ultra Gas Chromatography coupled with an ISQ Mass Spectrometer or equivalent model

Column	TG WAX/MSA (60 m × 0.25 mm i.d) or equivalent column	TG WAX/MSA (60 m × 0.25 mm i.d) or equivalent column
Film thickness	0.25 µm	0.25 µm
Oven temperature	50°C for 0.5 min 300°C at 5°C/min for 10 min	40°C for 2 min 250°C at 5°C/min 300°C at 30°C/min for 10 min
FID temperature	310°C	-
Injector temperature	280°C	250°C
Sample injection volume	1 µL	1 µL
Split ratio	100:1	1:20
Carrier gas	Nitrogen (0.30 mL/min)	Helium (1 mL/min)

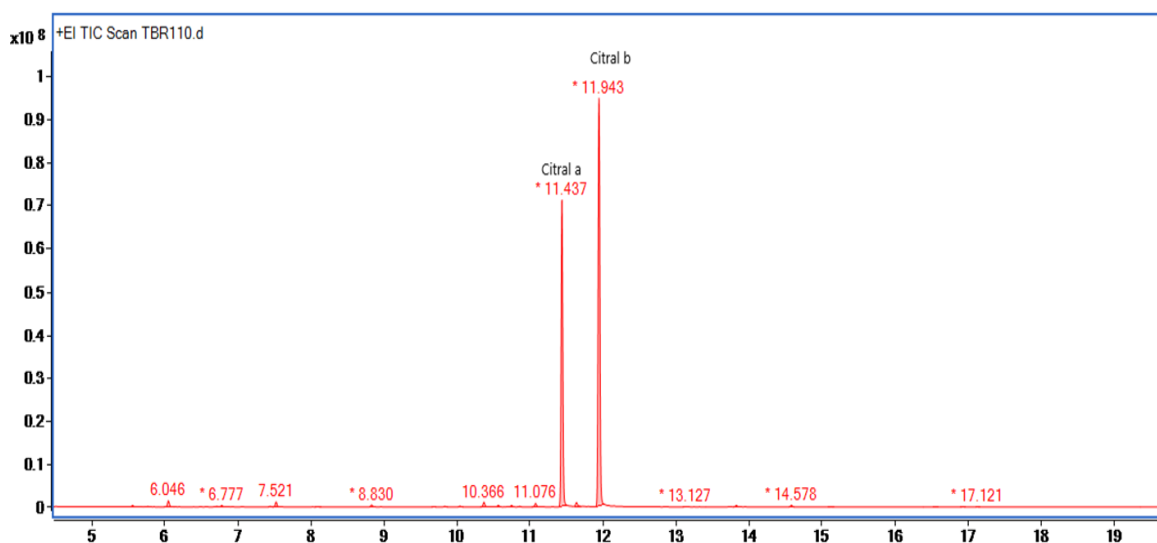
The compounds were identified by the comparison of retention time of the standard samples (Sigma Aldrich, Hi Media and other companies) with the test samples having same GC conditions and the percentages of the compounds were determined by the area normalization method. In GC–MS, mass spectra of the obtained peaks were identified by comparing with the NIST/ WILEY mass spectra library along with the reported literature and candidate varieties.

GC/MS analysis identified three unique registered lines (ICAR-NBPGR, New Delhi) and the representative chromatograph of the candidate varieties are below:

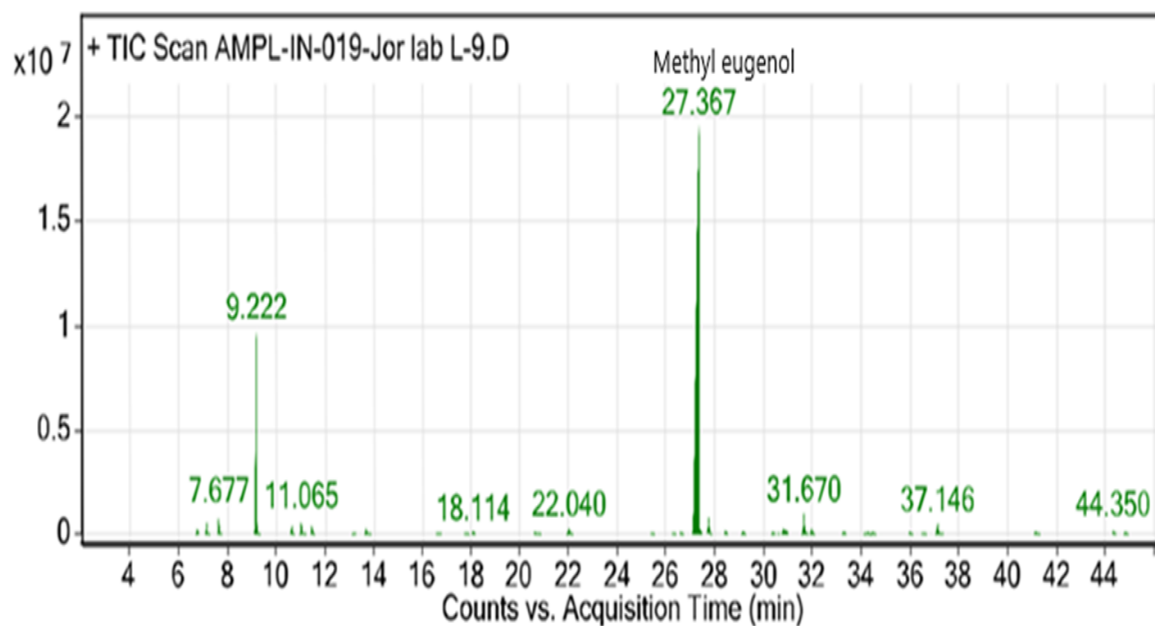
Citral rich: RRLJM 223 (JOR LAB L-8; INGR16020)

Methyl-eugenol rich: RRLJM 634 (JOR LAB L-9; INGR18037)

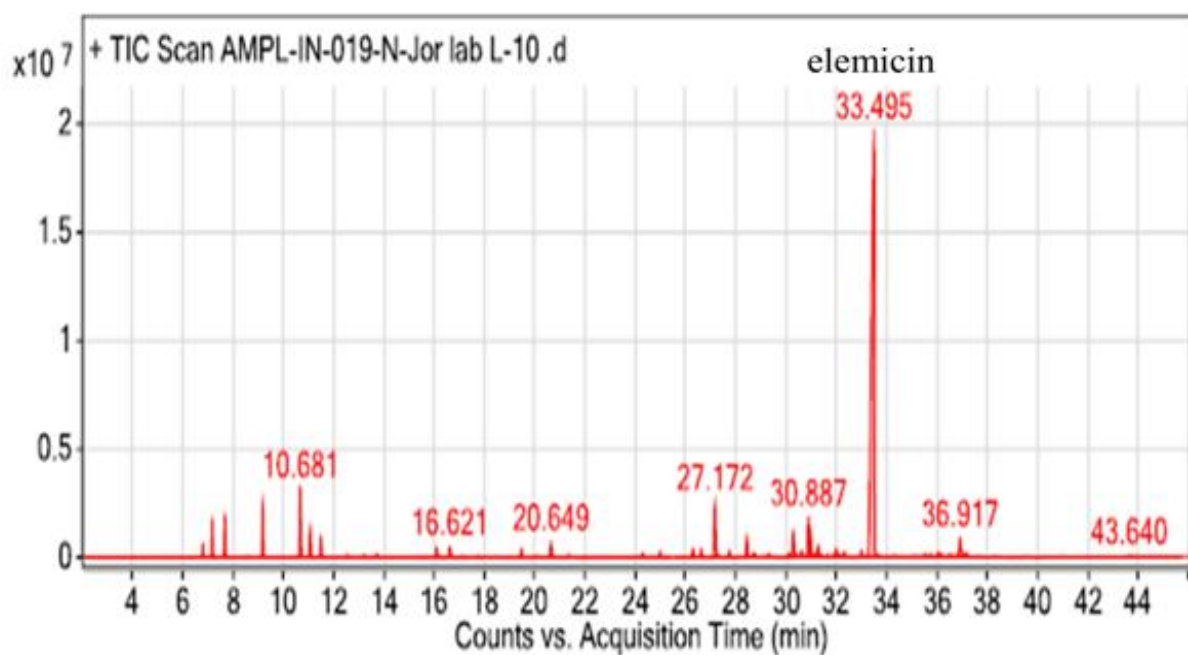
Elemicin rich: RRLJM 546 (JOR LAB L-10; INGR18039)



Chromatogram showing retention time of Citral a and Citral b



Chromatogram showing retention time of Methyl eugenol



Chromatogram showing retention time of Elemicin

IX. Working Group details

These test guidelines were developed by the National Core Committee in consultation with the Nodal officer DUS test centre CSIR-NEIST, Jorhat and Task Force (02/2023) constituted by the PPV&FR Authority.

Team of the Nodal centre

1. Dr Mohan Lal Principal Scientist (PBG), PI and Nodal Scientist
2. Dr Twahira Begum Scientist (PBG), Co-PI and Co-Nodal Scientist

The Members of the Task Force:

- | | |
|---|-------------------|
| 1. Dr. T. S. Mehra
Professor & Head
MAP Division, College of Horticulture and Forestry
(Central Agricultural University)
Pasighat- 791102, Arunachal Pradesh | Chairman |
| 2. Dr. P. Manivel
Head and Principal Scientist
ICAR-CTRI Regional Station Vedssandur
District: Dindugal- 624710, Tamilnadu. | Member |
| 3. Dr. R. K. Lal
Emeritus Scientist & chief Scientist
CSIR-Central Institute of Medicinal and Aromatic Plants
P.O-CIMAP, Near Kukrail Picnic Spot Lucknow-226015 | Member |
| 4. Dr. S. K. Shukla
Ex Chief Scientist, CSIR-NBRI Lucknow, UP | Member |
| 5. Dr. Sain Dass
Ex director, IIMR New Delhi | Member |
| 6. Dr. R. K. Gautam,
Principal Scientist, NBPGR New Delhi. | Member |
| 7. Dr. Mohan Lal
Principal Scientist (Plant Breeding & Genetics)
Agrotechnology Technology & Rural Development Division,
CSIR-North-East Institute of Science & Technology (CSIR-NEIST)
P.O.- RRL, Jorhat 785006, Assam | PI of DUS Project |
| 8. Shri R. S. Sengar
Deputy Registrar, PPVFRA, New Delhi | Member Secretary |

X. Name of DUS testing centre

Nodal centre	Co-nodal centre
CSIR-North-East Institute of Science & Technology (CSIR-NEIST), P.O.- RRL, Jorhat 785006, Assam.	CSIR-Central Institute of Medicinal and Aromatic Plants, P.O-CIMAP, Near Kukrail Picnic Spot Lucknow-226015