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Identification of a novel germplasm (Jor Lab L-9) of lemon grass (*Cymbopogon khasianus*) rich in methyl eugenol

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Abstract: *A new strain of Cymbopogon khasianus rich in methyl eugenol was identified and named Jor Lab L-9. It is stable for production of essential oil yield (average 0.81%; $\sigma^2di = 0.00$) and methyl eugenol (average 74.56%; $\sigma^2di = 0.06$). This variety was registered with ICAR-NBPGR, New Delhi, India, under registration number INGR-18037.*

Keywords: *Cymbopogon khasianus, essential oil quality, methyleugenol.*

INTRODUCTION

Species of the *Cymbopogon* genus are important and valuable medicinal and aromatic crops in the Poaceae family. They are cultivated in parts of Asia, America, and Africa (Lal 2018). Essential oil is extracted from *Cymbopogon* species by the steam distillation method, and this oil is used in perfumery, soap, cosmetics, floor cleaner and flavouring industries throughout the world (Dutta et al. 2017, Baruah et al. 2017). Different chemical constituents,*viz*., methyl eugenol, myrcene, geraniol, elemicin, citral, linalool, geranyl acetate, methyl isoeugenol, etc., are present in the essential oils of this genus (Dutta et al. 2016, Dutta et al. 2017). Among them, methyl eugenol, an important chemical constituent belonging to the phenylpropanoid group, is found in different spices, vegetables, and herbs (Linchtenstein and Casida 1963). This chemical can be converted to either elemicin or myristicin, a highly valuable compound (Koezuka et al. 1986). Methyl eugenol is used for flavour in baked confectionery products, non-alcoholic beverages, condiments, and hard and soft candy at a concentration level < 50 ppm (Hall and Oser 1965); in processed food; in perfumery; and in aromatherapy (Tan and Nishida 2012). A mixture of methyl eugenol and cue-lure at different concentrations in Male Annihilation Techniques (MAT) has been used to control pests in Taiwan and Okinawa through their effectiveness against *Bactocera dorsalis* and *Bactocera curcubitae* (Vargas et al. 2000). Methyl dopa, an important hypertensive medication, can be synthesized from methyl eugenol (Robinson and Barr 2006, Kumaran et al. 2013). The various uses of methyl eugenol in the flavouring, perfumery, and pharmaceutical industries and the lack of availability of a *C. khasianus* strain rich in this substance highlights the importance of identification, for the first time, of a high methyl eugenol (74.56%) rich variant from the *C. khasianus* germplasm collection. The genotype by environment interaction should always be evaluated for the release of any new variety (Agahi et al. 2020). Stability tests on the performance of this strain

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identified as rich in methyl eugenol have been conducted over the years in multiple locations.

BREEDING METHOD

Two hundred seventy-two (272) accessions of lemon grass were collected throughout the states of Northeast India (*viz*., Manipur, Assam, Meghalaya, and Nagaland) in the year 2013 and were planted, with three replications, on 8 November, 2013, in a randomized complete block design (RCBD) in a 4 × 3 m plot size, with plant-to-plant and row-to-row spacing of 60 × 60cm at the CSIR-NEIST, Jorhat experimental research farm in a selection trial. All the materials planted were authenticated by a taxonomist of the Botanical Survey of India, Shillong, Meghalaya (vide letter No.BSI/ERC/Tech/2015/448), and voucher specimens were prepared and kept at the Department herbarium of the CSIR-NEIST Jorhat. All the morphological and chemical data for all the planted germplasm were recorded in triplicates during 2013-14 and 2014-15 in selection trials (Table 1). The first harvest was made 4.5 months after planting, and then the crops were harvested at an interval of every 3 months.

After evaluation of the experimental data for two years, a methyl eugenol-rich genotype was identified, which was named Jor Lab L-9. Multi-location trials (MLT) were conducted for this genotype, along with the *C. khasianus* RRLJM-637 (IC 0626782) and *C. flexuosus* (Jor Lab L-2) check varieties, at four locations in Northeast India [*viz.*, Pasighat (Arunachal Pradesh), Imphal (Manipur), Jorhat (Assam), and Lakhmijan (Assam)] for three crop years (2015-16, 2016-17, and 2017-18). All the morphological and chemical data of the germplasm planted in each location were recorded using five replications, as per standard protocol (Lal et al. 2018). Stability analyses of the germplasm were analysed using the Eberhart and Russell (1966) model.

Essential oil was isolated using the Clevenger apparatus, in which 300g samples of leaves from each *C. khasianus* germplasm were hydro distilled for three and half hours in 2.5 L of water (Guenther 1950), with three replications. After extraction, the essential oil was dried over anhydrous Na_2SO_4 and was stored in glass vials at 4 ºC.

GC/FID analysis of the essential oil was performed using an Agilent Technologies gas chromatograph model no. 6890 fused with a HP-5MS capillary column (30 m × 0.25 mm i.d., 0.25 µm film thickness). GC/MS analysis was performed on an Agilent Technologies gas chromatograph coupled to a mass selective detector MSD 5975C using a fused silica capillary HP-5MS column (30 m × 0.25 mm i.d.; film thickness 0.25 µm). The components were identified by matching their mass spectra value to the Wiley mass spectral/NIST library, which was then confirmed by comparison with retention indices on the HP-5MS column (Khan et al. 2016). The concentration of the identified compounds was directly calculated from peak areas.

Stability analysis of eight essential traits, *viz.*, flowering plant height, vegetative plant height, number of tillers/bush, number of internodes, number of nodes, essential oil yield, herbage yield, and methyl eugenol (%) was performed using the Indostat statistical software, version 8.2. The Eberhart and Russel (1966) method was used to interpret the regression coefficient (bi), and deviation from the regression coefficient (σ^2di) was used to check stability.

PERFORMANCE CHARACTERISTICS

Different agronomical data were recorded for all the collected *C. khasianus* germplasm for two years (2013-14 and 2014-15) in selection trials, which led to identification of a methyl eugenol rich strain. The line identified achieved herbage yield of 29.50 tones ha^{-1} $year^{-1}$ and average essential oil yield of 0.81% (Table 2). It had previously been reported

Table 1. Range of different agronomic traits and essential oil percent in the collected *C. khasianus* germplasm

Sl.No.	Trait	Range
1	Vegetative plant height (cm)	69-143
2	Flowering plant height (cm)	91-250
3	Number of tillers $bush^{-1}$	87-92
4	Number of leaves	485-526
5	Length of lamina (cm)	56-73
6	Breadth of leaf (cm)	0.4-0.8
7	Length of basal sheath (cm)	12-34
8	Length of ligule (cm)	0.1-0.5
9	Length of auricle (cm)	0.09-0.1
10	Colour of leaf sheath	Brownish green
11	Number of nodes	4-12
12	Number of internodes	3-11
13	Diameter of nodes (cm)	1-2.20
14	Diameter of internodes (cm)	0.50-1.20
15	Length of internodes (cm)	16-27.70
16	Length of spike (cm)	78.20-110.50
17	Number of spikelets	7-12
18	Length of awn (cm)	0.70-1.10
19	Flowering time	last week of October to December
20	Herbage Yield (tones $^{-1}$ ha^{-1} $year^{-1}$)	18.3 to 36.4
21	Essential oil (%)	0.5-0.85
22	Methyl eugenol (%)	2-77

that fresh leaves of *C. khasianus* through hydro distillation has essential oil content of 0.70% (Choudhury 1995).

The GC/FID and GC/MS analysis of Jor Lab L-9 essential oil from multi-location trials of Jorhat in the 2017-18 crop year showed a total of 25 peaks, in which methyl eugenol was the major compound, with a concentration of 74.23%, followed by β-myrcene (14.28%), elemicin (1.65%), cis-α-bergamotene (1.19%), and β-pinene (1.11%) (Table 3). The average methyl eugenol content of the multi-location trial was found to be 74.56%. However, a study on essential oil of *C. khasianus* from NE India showed the presence of geraniol (83%) as the major compound (Lal et al. 2019). Moreover, a recent study was also conducted on *C. khasianus* collected from the Meghalaya region of Northeast India, where elemicin (70%) was the major component (Lal et al. 2018),which is different from our study.

Table 2. Agronomic traits and essential oil percent of the methyl eugenol-rich genotype (Jor Lab L-9) of *C. khasianus* (average of three crop years in four locations)

Sl.No.	Trait	Quantitative data
1	Vegetative plant height (cm)	109.83±1.81
2	Flowering plant height (cm)	230.28±4.40
3	Number of tillers bush^{-1}	95.53±3.38
4	Number of nodes	13.20±0.66
5	No. of internodes	12.17±0.82
6	Herbage Yield (tons ha^{-1} year^{-1})	29.50±0.90
7	Essential oil (%)	0.81±0.01
8	Methyl eugenol (%)	74.65±0.19
9	Flowering time	October to December

The germplasm identified (Jor Lab L-9) was planted in four locations of Northeast India (Jorhat, Imphal, Pasighat, and Lakhimijan). Since no methyl eugenol-rich variety has been available until now, the RRLJM-637 variety, with methyl

Table 3. GC/MS analysis of essential oil of *C. khasianus* (Jor Lab L-9) grown in Jorhat in multi-location trials

S. No.	Compound	Retention time	Area (%)	RI[a]	RI[b]	Identification method
1	α-pinene	6.802	0.29	912	917	1,2,3
2	Camphene	7.179	0.70	923	933	1,2,3
3	β-pinene	7.677	1.11	957	964	1,2,3
4	β-myrcene	9.222	14.28	976	981	1,2,3
5	α-terpinene	10.676	0.61	1011	1016	1,2
6	Limonene	11.065	0.88	1022	1031	1,2,3
7	1, 8 cineole	11.494	0.54	1032	1036	1,2,3
8	Trans-β-ocimene	13.21	0.05	1043	1050	1,2
9	α-terpinolene	13.714	0.36	1083	1097	1,2
10	Linalool	16.621	0.06	1099	1100	1,2,3
11	Borneol	17.765	0.06	1161	1165	1,2,3
12	α-terpineol	18.114	0.09	1182	1190	1,2
13	Nerol	20.643	0.11	1223	1229	1,2,3
14	Bornyl acetate	22.04	0.42	1282	1286	1,2
15	α-cubenene	25.45	0.05	1340	1345	1,2
16	α-copaene	26.302	0.05	1371	1378	1,2
17	**Methyl eugenol**	**27.367**	**74.23**	**1392**	**1402**	1,2,3
18	Cis- α-bergamotene	27.762	1.19	1428	1436	1,2
19	β-caryophyllene	28.454	0.19	1446	1451	1,2,3
20	α-humulene	29.169	0.15	1452	1456	1,2
21	γ-Muurolene	30.949	0.18	1472	1478	1,2
22	Elemicin	31.67	1.65	1561	1566	1,2,3
23	Caryophyllene oxide	31.984	0.36	1583	1589	1,2,3
24	Epicubenol	33.306	0.10	1622	1627	1,2
25	Cadinol	37.146	0.89	1672	1679	1,2
Total identified (%)				98.60		
Monoterpenes (%)				19.56		
Sesquiterpenes (%)				3.16		
Phenylpropanoid (%)				75.88		

[a] RI =Experimental retention index; [b] RI =Literature retention index; * Identification methods: 1. Comparison of retention indices with the literature; 2. Comparison of the mass spectra with the mass libraries; 3. Standards, by comparing retention time with the same GC condition.
** Khan et al. (2016).

eugenol content of 25%, and Jor Lab L-2, rich in citral, were planted to compare their data with the methyl eugenol-rich variety identified. The multi-location trials were conducted and recorded for the 2015-16, 2016-17, and 2017-18 crop years (Table 4). Stability analysis of the identified variety along with the check varieties revealed maximum means values of Jor Lab L-9 for all the traits, except vegetative plant height (Table 5). Jor Lab L-9 has a *bi* > 1 for the traits herbage yield, methyl eugenol (%), number of nodes, and number of internodes. The smallest deviation from regression was seen in the essential oil yield trait (σ^2di = 0.00), followed by methyl eugenol content (σ^2di = 0.06), indicating that it is a stable and desirable trait. Eberhart and Russell (1966) stated that a genotype is stable if it has high mean performance, a regression coefficient equal to 1(*bi* = 1), and deviation from regression (σ^2di) as low as possible. Similarly, the traits of herbage yield (σ^2di = 0.41), number of nodes (σ^2di = 0.19), and number of internodes (σ^2di = 0.46) were found to maintain moderate consistency in the respective environment (Tables 5 and 6). The stability and high yield of germplasm forms the basis of a successful breeding programme, leading to development of a superior progeny (Munda et al. 2020).

Table 4. Agronomic and essential oil quality data of the *C. khasianus* genotype Jor Lab L-9 under multi-location trials in the 2015-16, 2016-17, and 2017-18 crop years (pooled data)

Sl. No.	Variety	Location	Vegetative plant height (cm)	Flowering plant height (cm)	Number of tillers bush^{-1}	Number of nodes	Number of internodes	Herbage Yield (tonnes ha^{-1} year^{-1})	Oil % (v/w)	Methyl eugenol (%)	Major oil constituent
1	Jor Lab L-9	Jorhat	106.81	236.27	92.33	12.07	11.13	29.86	0.81	74.98	Methyl eugenol
		Imphal	108.66	219.55	95.60	12.47	11.33	28.78	0.81	72.77	
		Pasighat	111.56	238.17	95.13	14.67	13.73	29.57	0.82	74.60	
		Lakhmijan	112.29	227.12	99.07	13.60	12.47	29.81	0.81	75.90	
		Avg	109.83	230.28	95.53	13.20	12.16	29.51	0.81	74.56	
2	RRLJM-637 (IC0626782)	Jorhat	104.17	177.65	55.13	11.73	11.07	22.16	0.38	25.75	Methyl eugenol
		Imphal	103.05	189.09	59.87	12.00	10.93	21.59	0.38	25.99	
		Pasighat	105.94	192.92	58.40	14.20	13.20	21.55	0.39	25.87	
		Lakhmijan	109.87	202.55	61.00	12.73	11.73	21.99	0.39	25.40	
		Avg	105.76	190.55	58.60	12.66	11.73	21.83	0.39	25.76	
3	Jor Lab L 2	Jorhat	106.04	182.55	52.40	12.40	11.33	21.40	0.37	4.19	Citral
		Imphal	110.72	192.03	55.20	13.60	13.13	21.81	0.43	3.48	
		Pasighat	114.32	190.43	61.00	12.87	12.40	20.70	0.40	3.42	
		Lakhmijan	113.81	192.89	55.20	12.40	11.87	20.87	0.40	3.83	
		Avg	111.22	189.48	55.95	12.81	12.18	21.20	0.40	3.73	
		SE (m)	1.64	13.43	12.77	0.16	0.15	2.67	0.14	20.93	
		CV	2.60	11.43	31.60	2.16	2.11	19.13	44.93	104.51	

Table 5. Estimation of mean and stability parameters of vegetative plant height, herbage yield, essential oil yield, methyl eugenol (%), and flowering plant height in MLT trials

Genotypes	Vegetative plant height			Herbage yield			Essential oil yield			Methyl eugenol (%)			Flowering plant height		
	μ	βi	σ^2di	μ	βi	σ^2di	μ	βi	σ^2di	μ	βi	σ^2di	μ	βi	σ^2di
Jor Lab L-9	109.8	0.57	5.82**	29.51	1.43	0.41	0.81	0.57	0.00	74.56	1.13	0.06	230.3	-0.12**	107.15***
RRLJM-637	105.8	1.19	16.57***	21.83	0.93	0.42	0.39	0.33	0.00	25.76	1.17	1.43***	190.6	0.89	279.89***
Jor Lab L 2	111.2	1.23	29.97***	21.20	0.64	0.23	0.40	2.10	0.00	3.73	0.22*	-0.01	189.5	2.23*	267.14***
Population mean	108.9			24.18			0.53			34.68			203.4		

* Significant at *P*=0.05, ** Significant at *P*=0.01, *** Significant at *P*=0.005.

Table 6. Estimation of mean and stability parameters of number of tillers bush^{-1}, number of nodes, and number of internodes in MLT trials

Genotypes	Number of tillers bush^{-1}			Number of nodes			Number of internodes		
	μ	βi	σ^2di	μ	βi	σ^2di	μ	βi	σ^2di
Jor Lab L-9	95.53	0.44	21.36 ***	13.20	1.54	0.19	12.16	1.50	0.46
RRLJM637	58.60	0.70	26.09 ***	12.66	1.07	0.05	11.73	1.08	0.02
Jor Lab L 2	55.95	1.85	26.50***	12.81	0.39	0.29	12.18	0.41	0.64*
Population mean	70.03			12.89			12.02		

*Significant at *P*=0.05, ** Significant at *P*=0.01, *** Significant at *P*=0.005.

GERMPLASM REGISTRATION AND DISTRIBUTION

This is the first report of a methyl eugenol-rich strain of lemon grass identified through a pure line plant breeding method. The variety was registered with ICAR-NBPGR, New Delhi (vide registration number INGR-18037), and planting material is available for commercial cultivation. Two agro-based industries have been created for commercial cultivation, and planted area is more than 42 ha in different regions of India.

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