Medicinal Plants
Vol. 10 (3), September 2018, 203-209

doi : 10.5958/0975-6892.2018.00033.3

IndianJournals.com
A product of Diva Enterprises Pvt. Ltd.

Research Article

A new chemotype of palmarosa [*Cymbopogon martini* (Roxb.) W. Watson] identified from 'The Aravali Range' of Rajasthan, India

G.R. Smitha[1,2]* and H.L. Dhaduk[3]

[1]*Scientist (Horticulture), ICAR-Directorate of Medicinal and Aromatic Plants Research, Boriavi, Anand, Gujarat, India*
[2]*Scientist, ICAR-Indian Institute of Horticultural Research, Hessaraghatta, Bengaluru, Karnataka, India*
[3]*Associate Professor, Department of Genetics and Plant Breeding, B.A. College of Agriculture, Anand Agricultural University, Anand-388110, Gujarat, India*

Received: May 18, 2018; Accepted: July 04, 2018

ABSTRACT

An exploration was conducted to Aravali range of Udaipur, Rajasthan wherein wild palmarosa covering the entire hills was noticed. Observation of the plants revealed entirely different aroma compared to cultivated palmarosa. Hence, detailed investigation was carried out in this genotype for morphological, yield and quality parameters with released variety of palmarosa vir. PRC-1 as check. Two years observation revealed that though the Udaipur chemotype is short and bushy, it recorded significantly highest dry biomass yield, essential oil content and essential oil yield (0.67 kg/pt, 1.04% and 6.97 mg/pt, respectively) compared to cultivated palmarosa (0.52 kg/pt, 0.93% and 4.8 mg/pt, respectively). Further, morphological characterization carried out based on collar, auricle and ligule of the leaf and involucral bract of the inflorescence revealed no significance variation between these two accessions. However, GC/MS analysis of the essential oils of these two accessions showed entirely different spectra of chemical compounds. The major component of essential oil in cultivated palmarosa is geraniol (78.29%) and geraniol acetate (6.16%) whereas, it's Udaipur chemotype contains limonene epoxide (22.1%), trans carvyl formate (17.31%), limone-10-ol (17.21%), d-limonene (12.95%), dihydro linalool acetate (9.3%), limonene epoxide (5.31%) as the major components. As the newly identified chemotype is wildly distributed in Aravali hills of Udaipur, it can be commercially utilized for its essential oil, once its industrial utility is established and it can also be utilized in palmarosa breeding programme.

Keywords: Geraniol free, essential oil profiling, morphological characterization, *Rosha* grass

INTRODUCTION

Palmarosa (*Cymbopogon martini* (Roxb.) W. Watson belongs to family Poaceae with chromosome number of 2n=2x=20 (Bir, 1990). It is popularly known as *Russa* / *Rosha* grass, yields aromatic oil of economic importance and is accredited as the modern cash crop in India. Amongst *Cymbopogon* species, palmarosa is most widely adapted to diverse edapho-climatic conditions and occurs widely in the tropics and subtropics (Sangwan *et al*., 2001). The flowering tops and leaves yield essential oil which is rich in geraniol, geranyl acetate and linalool. This essential oil is used worldwide in the manufacturing of soap, perfumery and cosmetics because of resemblance of its aroma with that of rose (Sahu *et al*., 2000; Verma *et al.,* 2010). It also exhibits antifungal and antibacterial properties. This crop is native to India and is naturally grown in the forests of Maharashtra, Madhya Pradesh, Andhra Pradesh, Karnataka, Tamil Nadu and Uttar

*Corresponding author e-mail: smithagingade@gmail.com

Pradesh. Apart from India, it is also cultivated in Brazil, Paraguay, Madagascar, Guatemala and Indonesia (Sahu *et al*., 2000; Singh and Kumar, 2000; Verma *et al.,* 2010). India is a major producer and supplier of Palmarosa oil to the world market and about 44 tons of Palmarosa oil was exported to about 19 countries, including the United States of America, France, and the Philippines during 2009-2010 (Anon., 2010).

Palmarosa is a highly cross-pollinated crop; hence lot of variations are observed in relation to plant height, number of tillers, stem girth, color of stem and inflorescence, leaf sheath, node, crop maturity and oil content. Main constituent of palmarosa essential oil is geraniol, which determines oil quality and market rate. But, during a recent exploration to Aravali range of Udaipur, palmarosa plants covering the entire hills was noticed (Figure 1). Close observation of these plants revealed presence of entirely different aroma from that of usual palmarosa. Hence, it was collected and cultivated to study growth, essential oil content and composition.

Figure 1: Aravali hills covered fully with palmarosa chemotype at Udaipur, Rajasthan

MATERIAL AND METHODS

Plant Materials and Research Site

The palmarosa plant at seed setting stage was collected from a wild population of Aravali hills of Udaipur (25° N; 73°5' E), Rajasthan. From this selection, seedlings were raised in the nursery beds during monsoon (June, 2009) along with seedlings of palmarosa var. PRC-1. When the seedlings were one month old, 200 seedlings each from individual selection were transplanted in to the main field at a spacing of 60×60 cm in separate plots at ICAR-Directorate of Medicinal and Aromatic Plants Research, Boriavi, Anand, Gujarat, India (22° 35' N; 72° 55' E).

Morphological Traits

The morphological characterization of variety PRC-1 and Udaipur chemotype was recorded using stereo zoom Olympus SZ40 microscope. The various characters *viz.*, collar, auricles, ligules and bracts were observed for their presence and size. The images of leaf and inflorescence were taken by using Canon 1000 D camera.

Recording Growth and Yield Parameters

During the initial period of establishment the growth was very slow and the first harvest was possible after eight months of transplanting. The subsequent harvests were done at 4-5 months interval depending upon the growth at different seasons. The crop was harvested at full flowering stage by cutting the individual clump 15 cm above the ground and allowed the clump for ratooning. Fresh weight of the whole herb was recorded using electronic balance and a sample of herb was dried at 105 ºC for ~ 72 h in order to measure their dry matter content. In total, five harvests were taken over a period of two years. At each harvest, observations on ten randomly selected plants in both the types with respect to growth parameters (plant height and number of tillers), yield parameter (dry weight of the plant) and quality parameters (essential oil content and oil yield) were recorded.

Isolation of Essential Oils

The shade dried aerial parts from each plant including leaves, stem and inflorescence (200g each) were hydro-distilled in Clevenger type apparatus for 3 h. The distillate was extracted with diethyl ether, the ethereal layer was dried over anhydrous sodium sulphate and ether distilled off on gently heated water bath. The oil content per plant was expressed as % w/w on dry weight basis. The essential oil yield per plant was estimated by multiplying oil content per plant and its dry weight and expressed as ml/plant.

Gas Chromatography-mass Spectrometry (GC-MS)

Analysis of the volatile oils was carried out on a GC/MS (Focus-PolariQ) Benchtop Ion Trap Mass spectrometer equipped with a ZB-5 capillary column (30 m x 0.25 mm i.d., film thickness 0.25 mm). Chromatographic conditions were as follows: injector temperature was 200ºC, helium as carrier gas at a flow-rate of 1 ml/min; injection volume was 1.0 ml (1µl oil in 1ml); respectively. The column temperature was held at 45º C for 5 min and programmed at 4º C /min to 200º C and held for 1 minute with split flow (1:70). The MS transfer line and source temperatures were 250º C and 200º

C, respectively. The GC column was coupled directly to Polaris-Q quadrupole ion trap mass spectrometer in EI mode at 70eV with the mass range of 40-500 a.m.u at 1 scan/s. The geraniol content was identified by mass spectra and their identity was confirmed by comparing their mass spectra with Mass Spectral Library (Ver. 2, 2005) and published literature.

Statistical Analysis

The analysis of variance was done in complete randomized block design for various observations recorded using statistical software SAS 9.2 (Anon., 2008). Comparison of growth, yield, essential oil content, essential oil yield and chemical compositions were made amongst the palmarosa and its chemotype was done for average of five harvests. The results were presented at 5% level of significance (P = 0.05). The LSD values were calculated to compare the various treatment means.

RESULTS AND DISCUSSION

Variety PRC-1 and Udaipur chemotype were compared for morphological, growth, yield and essential oil parameters for an average of five harvests (Table 1; Figure 2) and the results are interpreted hereunder.

Morphological Characterization

The morphological characterization of the plants of variety PRC-1 and Udaipur chemotype was carried out based on collar, auricle and ligule of the leaf and involucral bract of the inflorescence. It was apparent from the images that no significant variation existed between two collections studied (Figure 3). The collar region of leaf was found divided, auricle rudimentary with membranous ligule in the variety PRC-1 and Udaipur chemotypes. The involucral bracts range in length from 2.5- 3.0 mm in PRC-1 and 1.5 to 2.0 mm in Udaipur chemotype. The variation in lengths of involucral bracts between PRC-1 and Udaipur chemotype are meager (Figure 3). Based on the above fact it is evident that the Udaipur chemotype is also Palmarosa, *Cymbopogon martini* (Roxb.) W. Watson.

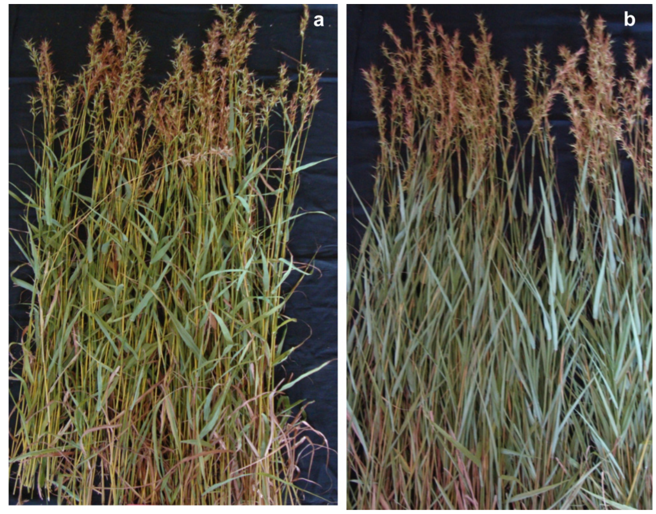

Figure 2: Close-up view of (a) variety PRC-1 and (b) Udaipur chemotype

Plant Growth and Yield Parameters

The comparison of growth, yield and essential oil content of these two accessions are presented in Table 1. Plant height was higher in PRC-1 (171.71 cm) compared to Udaipur chemotype (157.58 cm). Whereas, number of tillers/plant was maximum in Udaipur chemotype (202.49) compared to PRC-1 (154.38). This shows that the Udaipur chemotype is short and bushy compared to its commercial counterpart. The Udaipur chemotype also recorded significantly highest dry weight, essential oil content and oil yield (0.67 kg/plant, 1.04% and 6.97 mg/plant, respectively) compared to PRC-1 (0.52 kg/plant, 0.93% and 4.80 mg/plant, respectively). This means there was 28.8, 11.82 and 45% increase in dry biomass yield, essential oil content and essential oil yield, respectively in Udaipur chemotype compared to cultivated palamrosa.

Essential oil Profiling

The GC/MS analysis of these two collections showed entirely different spectra of chemical compounds (Table 2 and 3; Figure 3). In PRC-1 essential oil, a total of 51 peaks were observed, of which sixteen major peaks accounting to 96.88

Table 1: Comparison of growth and yield parameters of variety PRC-1 with Udaipur chemotype (average of five harvests)

Varieties	Plant height (cm)	Number of tillers	Dry weight (kg/plant)	Oil content (%w/w on DWB)	Oil yield (mg/plant DWB)
PRC-1	171.71	154.38	0.52	0.93	4.84
Udaipur Chemotype	157.58	202.49	0.67	1.04	6.97
LSD±	5.52	16.80	0.067	0.054	1.644

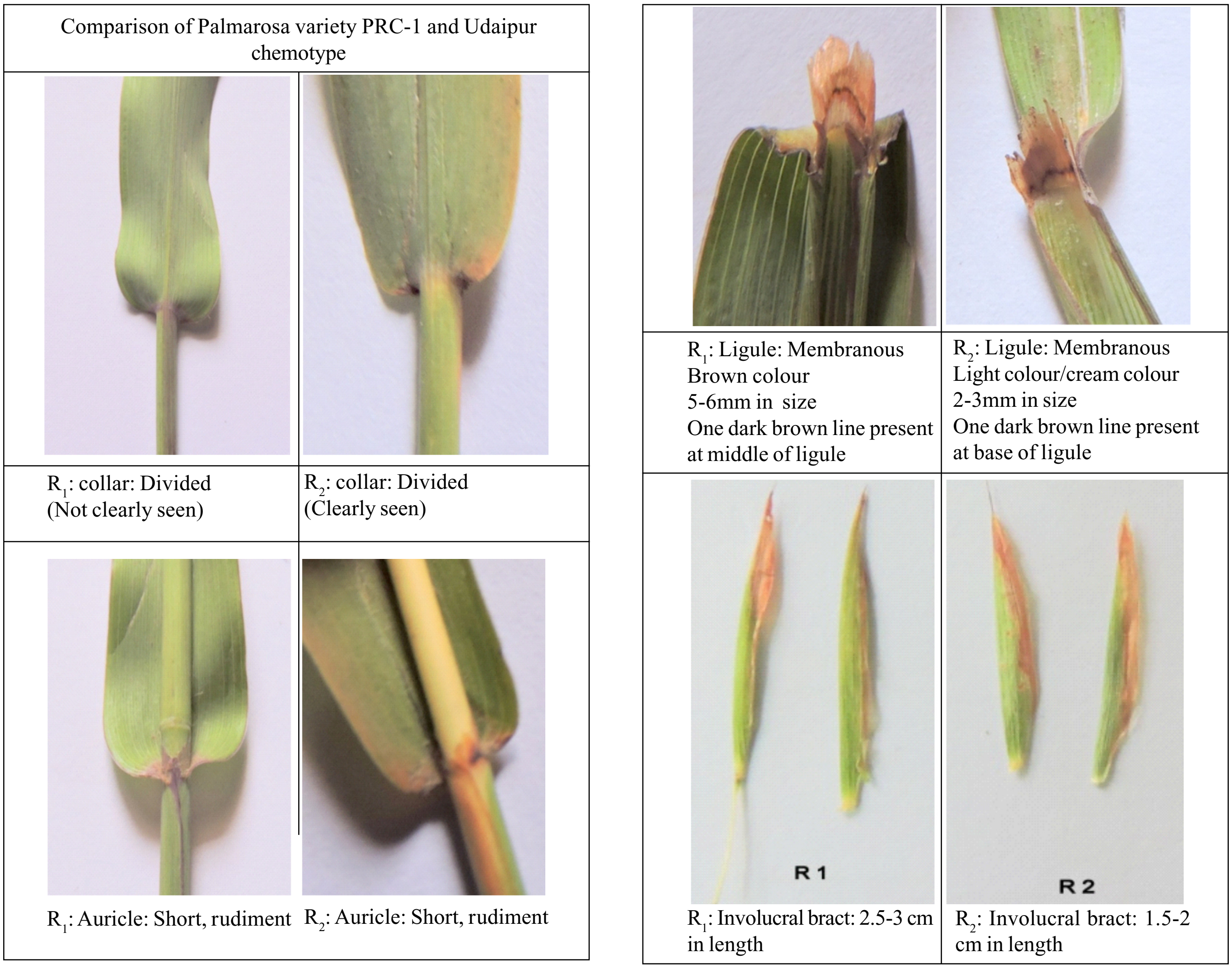

Figure 3: Morphological comparison of variety PRC-1 and Udaipur chemotype
R_1: Palmarosa, *Cymbopogon martini* (Roxb.) W. Watson grown at Directorate of Medicinal and Aromatic Plants Research, Boriavi, Gujarat
R_2: Palmarosa, *Cymbopogon martini* (Roxb.) W. Watson collected from The Aravali Range near Udaipur and established at Directorate of Medicinal and Aromatic Plants Research, Boriavi, Gujarat

% were identified (Table 2). This oil contained geraniol (78.29%) followed by geranyl acetate (6.16%), β-caryophyllene (3.64%), linalool (2.16%), delta-3-carene (1.82%), nirolidol (4.86%) and linalylformate (1.48%). Apart from this camphene, nerol, verbenal, citral, α-humulene, valencene, linalylformate, linalyl iso butyrate and hydroxyl caryophyllene were also reported in minor quantities.

The GC/MS spectra of Udaipur chemotype showed entirely different spectra of compounds (Table 3). In Udaipur chemotype, there were total 48 peaks, of which 34 major peaks accounting to 98.02% were identified. Here, major components identified were limonene epoxide (22.1%), trans carvyl formate (17.31%), limone-10-ol (17.21%), d-limonene (12.95%), dihydro linalool acetate (9.3%), limonene epoxide (5.31%), trans carvyl acetate (3.69%), d-carvone (1.94%) and dehydro linalool acetate (2.36%). Along with these minor quantities of camphene, p-cymene, p-α-dimethyl sterene, myrtenol, camphole, camphenelone, jasmone, verbenone, P-mentha-3, 8-Diene, acetophenone, 4-methyl, 4-pinanone, 2,3-Epoxy, perillyl alcohol, rose aldehyde, trans carvylacetate, 2-carene, geraniol, trans cinnamic acid, bornylformate, myrtenol, n-heptadecon, 2,6,6-trimethyl-2-cyclohexane, pyrazine, neryl acetate, isolongefolene, azulene and furan were noticed. Most interestingly, the geraniol

Table 2: GC/MS analysis of Palmarosa (variety PRC-1) essential oil

S.No.	Compound	Percentage
1	Camphene	0.14±0.015
2	Delta-3-carene	1.82±0.56
3	Linalool	2.16±0.55
4	Nerol	0.42±0.195
5	Verbenal	0.32±0.02
6	Geraniol	78.29±1.95
7	Citral	0.39±0.035
8	Geranyl Acetate	6.16±0.495
9	Beta Caryophyllene	3.64±0.74
10	Alpha Humulene	0.28±0.085
11	Valencene	0.30±0.17
12	Linalyl Iso Butyrate	0.37±0.14
13	Linalylformate	0.17±0.07
14	Hydroxy Caryophyllene	0.96±0.35
15	Nirolidol	4.86±0.6
16	Linalylformate	1.48±0.11

content which is a major component of palmarosa (70-90%) was found in traces (0.83%) in Udaipur chemotype.

In nature a wide range of diversity is observed in chemical composition of various essential oil yielding plants. Our finding is analogous to various other findings; wherein new chemotypes were identified with entirely different chemical spectrum. In *Ocimum gratissimum* L., a new geraniol rich chemotype (83.7–88.8%) was reported by Charles and Simon (1992). In Greece, Kofidis and Bosabalidis (2004) reported four different chemotypes of *Mentha spicata* based on their fragrance. The first chemotype was dominated by linalool (65.2% to 75.3%), second chemotype with a high carvone (35.2-68.4%) and dihydro carvone (5.4-21.5%), third chemotype with piperitone oxide (0.2-89.5%), and the fourth chemotype is rich in menthone, iso menthone and pulegone. Similarly, a new chemotype of *Mentha longifolia* (L.) with high content of piperitenone oxide (77.43%) and moderate amounts of germacrene D (3.7%) and 1,8 cineole (1.61%) has been reported (Venskutonis, 1996; Massimo Maffei, 2006). A new chemotype of *Scoparia dulcis* was reported from Taiwan, China and Thailand for scopadulciol and scopadiol content (Toshimitsu Hayashi *et al.,* 1993). *Cyperus rotundus* chemotype was identified in Hawaii, which had higher concentrations of patchoulenyl acetate and sugeonyl acetate (Komai and Tang, 2005).

Table 3: GC/MS analysis of Palmarosa Udaipur chemotype essential oil

S.No.	Compound	Percentage
1	Camphene	0.33±0.215
2	P Cymene	0.29±0.01
3	d-Limonene	12.95±0.34
4	p-Alpha dimethyl sterene	0.13±0.01
5	Myrtenol	0.18±0.015
6	Camphenelone	0.09±0.05
7	Limonene Epoxide	22.1±0.53
8	Dihydro Linalool Acetate	9.3±0.27
9	Jasmone	0.19±0.01
10	Verbenone	0.48±0.375
11	P-Mentha-3,8-Diene	0.30±0.155
12	Acetophenone, 4-Methyl	0.16±0.005
13	Trans-carvylformate	17.31±0.085
14	4-Pinanone, 2,3-Epoxy	0.10±0.02
15	Carvyl Acetate	0.76±0.04
16	Perillyl Alcohol	0.77±0.05
17	Rose Aldehyde	0.55±0.02
18	Trans Carvyl Acetate	3.69±0.025
19	Limonen-10-ol	17.21±0.425
20	2-carene	0.26±0.07
21	d-Carvone	1.94±0.08
22	Geraniol	0.83±0.255
23	Trans Cinnamic Acid	0.26±0.02
24	Bornyl Formate	0.15±0.045
25	Myrtenol	0.09±0.03
26	n-Heptadecon	0.16±0.015
27	Limonene Epoxide	5.31±0.76
28	Dehydro Linalool Acetate	2.36±0.56
29	2,6,6-trimethyl-2-cyclohexane	0.22±0.2
30	Pyrazine	0.37±0.065
31	Neryl Acetate	0.10±0.06
32	Isolongefolene	0.11±0.06
33	Azulene	0.90±0.05
34	Furan	0.13±0.06

The genus *Thymus* showed wide variability in the chemical polymorphism of essential oils for thymol and carvacrol contents. Thymol, citral, linalyl acetate and fenchone chemotypes were observed in Czechoslovakia (Stahl-Biskup, 1991), whereas geraniol (44.6%) and linalool (28.6%) chemotypes were found growing wild in Croatia

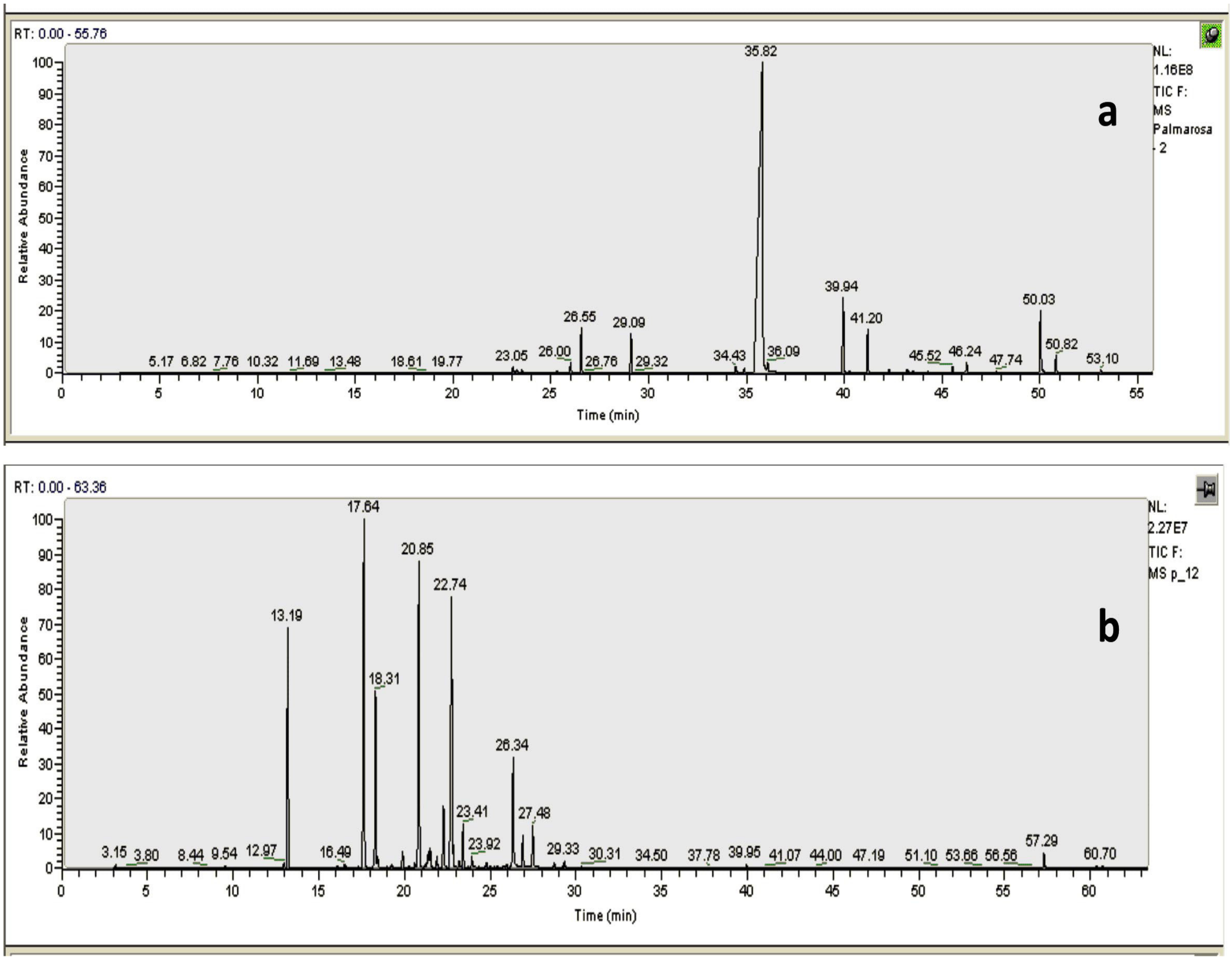

Figure 4: Total ion chromatograms of (a) variety PRC-1 (b) Udaipur chemotype

(Mastelic *et al.,* 1992; Mockute and Bernotiene, 2001). Variations in the chemical composition of Spanish populations of *Artemisia pedemontana* showed the presence of two chemotypes; *one was rich in* camphor and1,8-cineole, whereas, the another type with davanone as major component (Perez Alonso *et al.,* 2003). A rutin-free chemotype of *Hypericum perforatum* L. was identified from Italy by Martonfia *et al.* (2001). The chemical compositions of the oils show two chemotypes within *Valeriana wallichii.* The type-I was characterized by presence of maaliol (64.3%), viridiflorol (7.2%) and sesquiterpene hydrocarbons (19.2%). The type-II contained patchouli alcohol (40.2%), viridiflorol (5.2%), 8-acetoxy-patchouli alcohol (4.5%) and sesquiterpene hydrocarbons (34.5%) (Mathela *et al.,* 2005).

CONCLUSION

A new geraniol-free chemotype of palmarosa was identified, which resembled cultivated palmarosa in morphological characters but with entirely different chemical composition. As the cultivated palmarosa essential oil contains 70-90% geraniol and 7-8% geraniol acetate, its Udaipur chemotype on the other hand contains limonene epoxide (22.1%), transcarvyl formate (17.31%), limone-10-ol (17.21%), d-limonene (12.95%), dihydro linalool acetate (9.3%), limonene epoxide (5.31%) as the major components. The newly identified chemotype is wildly distributed in the Aravali range of Udaipur, which can be commercially utilized by mere distillation of its essential oil, and can be used by perfumery / industry for new product development. Considering the Udaipur chemotype's sturdy growth, high herbage and essential oil yield, it can also be utilized as one of the parent in palmarosa breeding programme for incorporating these characters in cultivated varieties.

ACKNOWLEDGEMENT

The authors are thankful to the Director, ICAR-Directorate of Medicinal and Aromatic Plants Research, Anand, Gujarat for providing facilities for this study. We are also Thankful to Dr. Dhara Gandhi, Department of Botany, M. S. University, Baroda for her help in characterization of palmarosa accessions. This work was supported by the Institutional funding from ICAR-Directorate of Medicinal and Aromatic Plants Research, Anand, Gujarat.

REFERENCES

Anonymous (2010). *Monthly statistics of foreign trade of India, exports including re-exports*. New Delhi, India: Ministry of Commerce and Industry.

Bir SS and Chauhan HS (1990). SOCGI plant chromosome number reports. *Journal of Cytology and Genetics*, 25: 322–323.

Charles DJ and Simon JE (1992). A New Geraniol Chemotype of *Ocimum gratissimum* L. *Journal of Essential Oil Research,* 4(3): 231–234.

Khoi Nguyen Xuan Dung, Marek Mardarowicz and Piet A Leclercq (2011). A new chemotype of *Hyptis suaveolens* (L.) Poit. from the Nghe an province, Vietnam. *Journal of Essential Oil Research,* 8(3): 315–318.

Kofidis G and Bosabalidis A (2004). Seasonal variation of essential oils in a linalool-rich chemotype of *Mentha spicata* grown wild in greece. *Journal of Essential Oil Research,* 16: 469–472.

Komai K and Tang C (2005). Seasonal and chemotype influences on the chemical composition of *Lantana camara* L., Essential oils from Madagascar. *Analytica Chimica Acta,* 545(1): 46–52.

Kustrak D and Martinis Z (1990). Composition of the essential oils of some *Thymus* and *Thymbra species*. *Flavour and Fragrance Journal*, 5: 227–231.

Maffei M (1988). A chemotype of *Mentha longifolia* (L.) hudson particularly rich in piperitenone oxide. *J. Flavour Fragrance,* 3(1): 23–26.

Martonfia P, Repcaka M, Ciccarellib D and Garbarib F (2001). *Hypericum perforatum* L. - chemotype without rutin from Italy. *Biochemical Systematics and Ecology,* 29: 659–661.

Mastelic C, Grzunov K and Kravar A (1992). The chemical composition of terpene alcohols and phenols from the essential oil and terpene glycoside isolated from *Thymus pulegioides* L. grown wild in Dolmatia. *RivistaItaliana EPPOS,* 3: 19–22.

Mathela CS, Tiwari Mamta, Subhash S Sammal and Chanotiya CS (2005). *Valeriana wallichii* DC, a new chemotype from northwestern Himalaya. *Journal of Essential Oil Research,* 17(6): 672–675.

Mockute D and Bernotiene G (2001).The α-terpenyl acetate chemotype of essential oil of *Thymus pulegioides* L. *Biochemical Systematics and Ecology,* 29: 69–76.

Perez-Alonso MJ, Velasco-Negueruela A, Pala-Paul J and Sanz J (2003). Variations in the essential oil composition of *Artemisia pedemontana* gathered in Spain: chemotype camphor-1,8-cineole and chemotypedavanone. *Biochemical Systematics and Ecology,* 31(1): 77–84.

Sahu S, Debata BK and Patnaik K (2000). Palmarosa and its improvement for geraniol production. *Journal of Medicinal and Aromatic Plant Science,* 22: 253–262.

Sangwan RS, Farooqi AHA, Fatima S and Sangwan NS (2001). Regulation of essential oil production in higher plants. *Plant Growth Regulation,* 34: 3–21.

Stahl-Biskup E (1991). The chemical composition of Thymus oils: a review of literature. *Journal of Essential Oil Research,* 3: 61–62.

Venskutonis (1996). A chemotype of *Mentha longifolia* L. from Lithuania rich in piperitenone oxide. *Journal of Essential Oil Research,* 8(1): 91-95.

Verma SK, Kumar B, Ram G, Singh HP and Lal RK (2010). Varietal effect on germination parameter at controlled and uncontrolled temperature in Palmarosa (*Cymbopogon martinii*). *Industrial Crops and Products,* 32: 696–699.