



ISSN (E): 2320-3862
ISSN (P): 2394-0530
www.plantsjournal.com
JMPS 2021; 9(5): 14-23
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Received: 09-06-2021
Accepted: 17-08-2021

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Selection parameters associated with essential oil yield comprise genetic and chemical diversity and the morphological expression of photosynthetic efficient agronomical traits in Vetiver (*Chrysopogon zizanioides* Roberty)

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Abstract

Using Mahalanobis D²-statistics and eleven of the most economically important features, the nature and extent of genetic divergence in eighty vetiver genotypes were evaluated. The vetiver underwent Mahalanobis D² analysis, which found a significant level of variety. Nine clusters were formed from the genotypes. Cluster I had the most genotypes (56), followed by cluster II (6), III (5), IV (3), V (3), VI (2), VII (2), VIII (2), and IX (2), with one in cluster IX. Cluster III genotypes had the highest average divergence ($\bar{D}^2=52.51$), followed by cluster IV ($\bar{D}^2=49.59$) and cluster VII ($\bar{D}^2=45.46$). Cluster III and VII ($D^2=85.52$), Cluster IV and Cluster VII ($D^2=81.04$), and Cluster IV and IX ($D^2=73.11$) had the highest inter-cluster distances, implying that the genotypes found in these clusters could be utilized for future breeding programmes. The significant contributors to genetic divergence were α -vetivone, essential oil yield, photosynthetic rate/net CO₂ assimilation rate, and transpiration rate, respectively. These varieties, such as Nos. 70, 25, 60, 15, 6, and 3 can be used to create commercial vetiver varieties.

Keywords: co-cultivation, genetic diversity, genetic distance; essential oil, efficient photosynthetic traits

1. Introduction

The vetiver (*Chrysopogon zizanioides* Roberty), a member of the Poaceae family, is native to India and grows wild in nearly every section of the country. The roots of vetiver are the source of the world-famous 'Khus' oil, which is highly prized in the essential oil industry (Hussain *et al.*, 1994; Pareek, 1994) [20]. It may be grown on saline and Sodic soils and sandy, wet and sloppy lands. Only the former of the two species found in India, *Vetiveria zizanioides* and *Vetiveria lawsonii* syn. *V. Nemoralis* has commercial importance due to its oil's high class fragrance value, which has been known since ancient times (Virmani and Datta, 1975; Lal *et al.* 2018a,b; Lavania, 2000) [23, 11, 16]. Vegetables grow abundantly in the wild in Uttar Pradesh, Bihar, Rajasthan (northern states), Southern and peninsular India, and are also grown on a small basis in Andhra Pradesh, Tamil Nadu and Kerala (Lal *et al.* 2018 c, d; Lal *et al.*, 1997a; Lal *et al.*, 1997b and Lal *et al.*, 1999; Singh *et al.*, 2019; Lal *et al.*, 2020a,b) [4, 13, 14, 15, 9]. The quality of vetiver oil, notably produced in north India, is regarded as the greatest in the world. Haiti, Indonesia and India are the top producers of vetiver oil. Vetiver is also planted in over a hundred nations for its environmental benefits, such as soil and water conservation and carbon sequestration. Essential oil yield in the world is expected to be between 300 and 350 tonnes per year. Annual consumption and demand are expected to rise even more. India produces roughly 100 tonnes of oil per year, which is insufficient to meet our domestic demand for oil for the perfumery, masticatory, attar and soap industries. Due to the strong demand for vetiver essential oil, farmers in north India have recently begun large-scale vetiver farming.

This crop has a lot of natural genetic variability. Genetic variability in vetiver genotypes is a precursor to possible crop improvement for various economic variables. Thus it's worth looking into the CSIR-Central Institute of Medicinal and Aromatic Plants, Lucknow, which maintains eighty indigenous and exotic vetiver collections representing twelve Indian states (Supplementary Table 1). CSIR-CIMAP also produced and released thirteen essential oil high producing vetiver cultivars for commercial cultivation, including KS-1, Sugandha, KS-2, Dharini, Gulabi, Kesari, CIM Vriddhi, CIMAP-KHUS 40 (induced tetraploid), G22, G15,

CIM-Khusinolika, CIM Samriddhi and CIMAP-Foragika (for fodder). There is a greater chance of developing high root and better quality essential oil-bearing cultivars by leveraging the genetic variability in this crop. With rising oil demand for the perfume and soap industries, Khus cultivation in India appears to be profit-driven. With a 25-30 kg/ha oil output, Khus cultivation yields a net return of approximately Rs. 1.5 lacs in 10-12 months. Furthermore, co-cultivation of Khus with wheat, paddy, lentil, peas, mint, basil, and other crops yields an extra benefit of roughly Rs. 30,000 per hectare (Lal *et al.*, 2018a,b; Singh *et al.*, 2019; Lal *et al.*, 2020a,b; Mishra *et al.*, 2020; Lal *et al.*, 2021a,b) ^[11, 9, 5].

Given the vast volumes of Vetiver oil still being imported, there is a pressing need to expand the area under khus cultivation by popularizing high oil production and early mature varieties of this crop. Because of the vetiver crop's economic importance and the availability of genetic diversity, efforts have been made to collect and characterize germplasm. This study aims to determine genetic divergence among genotypes and identify desirable genotypes for future vetiver crop exploitation.

2. Materials and methods

2.1 Plant materials

The materials for this study included 80 vetiver genotypes from indigenous and exotic collections of vetiver (*Vetiveria zizanioides* (L.) Nash ex Small syn. *Chrysopogon zizanioides* Roberty), representing twelve Indian states and four exotic collections from Indonesia, Haiti, Thailand and Reunion Island (Supplementary Table 1 and 2). They were evaluated at the CSIR-Central Institute of Medicinal and Aromatic Plants Research Farm, P.O. CIMAP, Lucknow, Uttar Pradesh 226 015. (India) in a randomized complete block design with three replications in two consecutive years: 2018-2019 and 2019-2020 on the Northern Indian plain's environment. Each row of 50×50cm slips was planted with 50×50cm plants. The plants received the standard intercultural operations, watering and fertilizer treatments of 100 kg N, 80 kg P₂O₅ and 40 kg K₂O/ha. Twelve months after transplanting, the plants' roots were uprooted and carefully collected. Hydro-distillation with a Clevenger-type apparatus was utilized to extract essential oil from the roots (Clevenger, 1928) ^[11]. Before analysis, the separated essential oils were stored at 4 °C. The volume (ml) of essential oil per 100 g of vetiver roots was used to calculate the oil content (%).

2.2 Morpho-metric observations

Morpho-metric observations were recorded for the eleven most economic traits, namely X1 = plant height (mt), X2-root yield/plot (g), X3 = essential oil content (%), X4 = essential oil yield (g/plot), X5 = photosynthesis rate/net CO₂ assimilation rate (μ mol m⁻² s⁻¹), X6 = transpiration rate (m mol m⁻² s⁻¹), X7 = stomatal conductance (m mol m⁻² s⁻¹). X8 = khusimol content (%), X9 = khusinol content (%), X10 = α-vetivone content (%), X11 = β-vetivone content (%) for D²-statistics and other allied genetic parameters.

2.3 Gas Chromatography, GC-Mass spectrometry and chemical analysis

A Varian CP 3800 system with an Elite-5 fused silica column (30 m 0.25 mm 0.25 μm) was used for GC analysis. The oven was configured to go from 60 °C at a rate of 3 °C per minute to 240 °C with a 2 minute hold time, then to a ramp rate of 10 °C with a final temperature of 310 °C and a final hold period of 1 minute. Temperatures in the injector and detector (FID)

were 300 °C, with a split ratio of 1: 100 and hydrogen as the carrier gas (1 ml/min with a continuous flow). Samples were prepared in dichloromethane and a 0.04 μL sample was injected manually in Split and Splitless injector.

GC/MS utilized a PerkinElmer Clarus 680 GC interfaced with SQ 8 C mass spectrometer as per the GC method. The ion source and transfer line temperatures were 250 °C, injector temperature 280 °C, the scan time of 0.39 sec with the inter-scan delay of 0.01 sec, helium as a carrier (1 ml/min. constant flow), and a split ratio of 1:100. Electron Impact Ionization (EI) was performed at 70 eV in the mass range from 40-450 AMU. The retention index was calculated using the same oven program using a homologous series of *n*-alkanes (C₆-C₂₈ hydrocarbons, Polyscience Corp. Niles IL). Compound identification was achieved by comparing two MS libraries (Turbo Mass NIST 2011 version 2.3.0 and Wiley registry of mass spectral data 9th edition), a reference guide on mass spectral data (Adams, 2017) and NMR experiments. The relative amounts of individual components were calculated based on GC peak areas without using correction factors.

2.4 Statistical analysis

Data were examined using statistical software version 3.0 in the PBGRC Division of the institute, for genetic diversity using D²-statistics (Panse and Sukhatme, 1967; Mahalanobis, 1936) ^[19, 17]. The genetic divergence among the test entries, including quantitative features, was assessed using D²-analysis. Tocher's approach, developed by Rao (1952) ^[21] was used to cluster all n(n-1)/2 D² values based on Singh and Chaudhary (1985).

3. Results and discussion

The pooled data analysis of variance for different traits found extremely significant variations among genotypes for all eleven traits, indicating that the material studied had a high degree of genetic variability and that genotypes were genetically diverse (Table 1). The eighty genotypes are grouped into nine clusters (cluster I, II, III, IV, V, VI, VII, VIII and IX (Table 2, 3, and 4; Figure 1-6). Among the clusters, the maximum number of accessions (56) were included in cluster I followed by cluster II (6), III (5), IV (3), V (3), VI (2), VIII (2) and IX (1), respectively (Table 2). Cluster III genotypes had the highest average divergence ($\bar{D}^2=52.51$), followed by cluster IV ($\bar{D}^2=49.59$) and cluster VII ($\bar{D}^2=45.46$). Cluster III and VII (D²=85.52), Cluster IV and Cluster VII (D²=81.04), and Cluster IV and IX (D²=73.11) had the highest inter-cluster distances.

The highest inter-cluster distance was observed between clusters III and VII (9.25) and the minimum inter-cluster distance between clusters I and II (5.43) (Table 3). The highest average (\bar{D}^2) values were recorded in cluster III (52.51), followed by cluster IV (49.59), V (45.53), VII (45.46), VIII (44.93), IX (44.84), VI (43.15), II (39.70), and I (34.50) in the study (Table 3). Cluster IX had the greatest mean value for X3 (2.09%). Cluster VII had the highest essential oil yield (8.94 g/plot), followed by cluster V with a 1.48 percent essential oil content of 6.25 g/plot. The tallest plant was found in cluster IX (1.69 mt). Cluster IV had the highest photosynthesis rate (X5) = 13.25, followed by Cluster V (11.88), Cluster II had the highest mean value for X6 (8.05), followed by cluster 7 (5.04), cluster III (4.80), and cluster I (4.56). Cluster IV had the highest value for the trait X7 (711.58) followed by cluster V (676.58), cluster VI (296.63) and cluster I (282.30), respectively.

Cluster VI had the highest khusimol content (23.59%),

followed by Cluster III (23.39%), Cluster V had the highest khusimol content (46.60%) and Cluster III had the highest khusimol content (43.53%). The highest root yielder was cluster IV (546.25 g/plot), clusters V and IX (502.08 g/plot). Cluster IX had the least root yield (412.71g/plot), and cluster VIII produced the least essential oil (0.61g/plot). Cluster III had the highest characteristic value (X9) of 5.61, followed by cluster VI (4.38), cluster IV (3.56) and cluster II (3.47). Similarly, for the trait X10, cluster VI had the highest value (4.18), followed by cluster II (3.23), cluster V (2.80), and cluster I (2.60). Cluster VIII had the lowest value for this feature, 0.66, which merely indicates a high level of genetic diversity (Table 4, Figure 1-5).

The current pattern of genotype categorization suggested that genetic diversity was not entirely tied to geographical variation. The current investigation thus highlighted the lack of a link between genetic diversity and geographic distance. Geographic diversity has traditionally been used as a proxy for genetic diversity. However, because this is an inferential criterion, it may not help quantify other populations. Desirable kinds can be chosen from the clusters based on the breeding program's goals. In the case of vetiver, these findings corroborate those of Virmani and Datta (1975) [23]; Pareek (1994) [20]; Husain *et al.*, 1984; Lal *et al.*, 1997a [13] and Lal, (2012) [3]; Lal *et al.*, (2017) [8]. Breeding programmes can efficiently use genetically varied types with important traits from the same or different regions.

Essential oil yield (g/plot) ranked I had the highest percentage contribution to the primary axis (λ_1 vector), followed by X6 = transpiration rate ($\text{mmol m}^{-2} \text{s}^{-1}$) ranked II, and X11 = vetivone content (%) ranked III. X7 = stomatal conductance ($\text{mmol m}^{-2} \text{s}^{-1}$) had the least contribution to genetic divergence. On the secondary axis (λ_2 vector), the highest contributor rated I was, followed by X9 ranked II and X11 ranked III, respectively. The X10 contributed the least to genetic diversity. On the other hand, the highest contributor was X11 (18.99%), followed by X7, 13.99% (II) and X6, 13.99% (III) (13.32%)

III. X1 was the least important factor in genetic divergence (2.69%) (XI) (Table 5).

The X9 (68.92%) had the highest coefficient of variation, whereas the trait X1 had the lowest (9.87%). The crucial difference between X7 and X1 was highest for X7 and lowest for X1. It was also observed that the vetiver accessions have a lot of genetic variation (Table 5). In the case of vetiver, our findings support those of Pareek (1994) [20]; Lal, (2012) [3] and Lal, *et al.* (2013) [10]; Lal *et al.* (2021a,b) [5]. As a result, a better selection criterion for improving the vetiver crop might be X4, X2, X5, X9 and X3, respectively. Because genetic improvement in any crop plant is solely dependent on the presence of significant genetic variability, we investigated genetic variability in eighty vetiver accessions to gain a better understanding of the variability, environmental conditions, and contributions of various traits to genetic diversity.

Nonetheless, in its never-ending quest to generate high-yielding vetiver genotypes/varieties, CSIR-CIMAP, Lucknow has just developed a new aroma variety of vetiver (CIM Samriddhi) from no. 70 (Figure 6 and 7) produces 35 kg of essential oil per hectare with >30% Khushilal and >19% Khusol, compared to 25 kg per hectare check variety CIM-Vridhi. The clonal selection breeding strategy was used to create the new variety. Superior clones within and between the clones were found based on their initial performance.

The selection for traits X4, X2, X1, X5 and X3 may provide good criteria for selection for essential oil yield. The presence of highly significant differences among the vetiver accessions studied indicates a significant amount of genetic variability in the eleven most economically important characters studied, implying that there is a good chance for genetic improvement in vetiver accessions and the development of superior varieties like CIM Samriddhi. X4, X2, X3, X5, X6, X7, X8, X9, X10 and X11 were the most important contributors. The No. 70, 25, 60, 15, 6 and 3 types can also make a commercial variety of vetiver (Table 6).

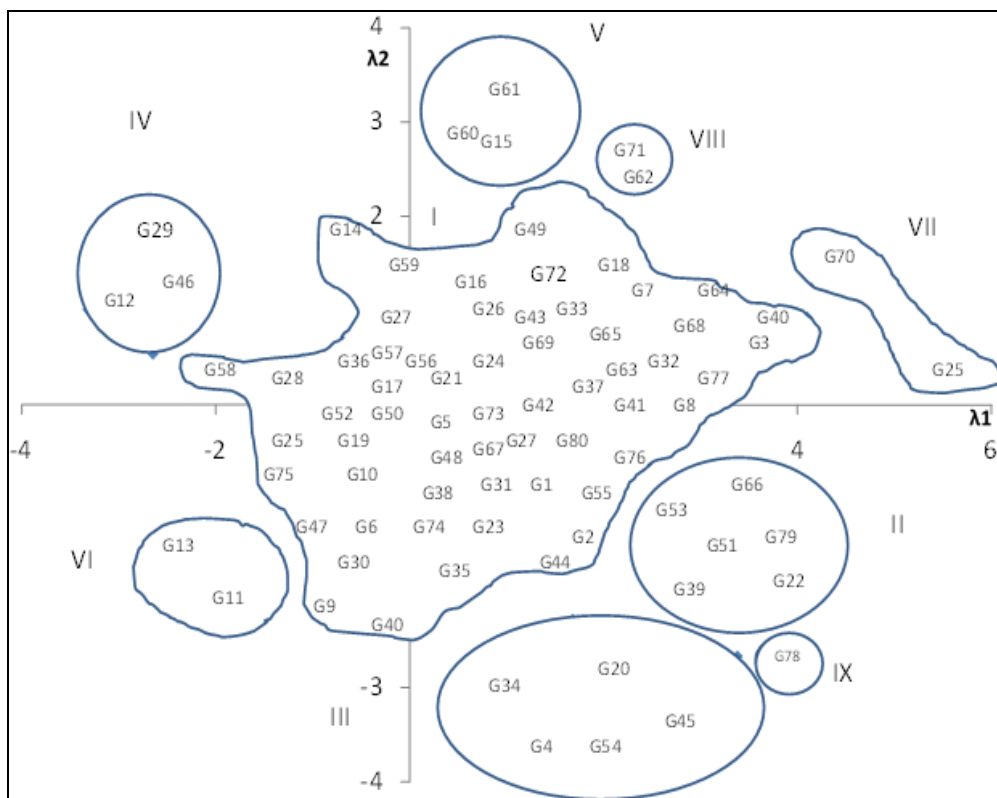


Fig 1: $\lambda_1 - \lambda_2$ chart showing the distributions pattern of vetiver genotypes

Table 1: Analysis of variance (ANOVA) for the eleven traits of the vetiver for the eighty genotypes

Source of variation	df	Characters mean sum of squares										
		X1	X2	X3	X4	X5	X6	X7	X8	X9	X10	X11
Replications	3	0.03	9286.67	0.11	2.05	18.99	0.75	3404.00	16.09	200.32	0.91	0.21
Genotypes	79	0.05**	24111.95**	0.49**	9.97**	48.39**	7.26**	74843.90**	249.39**	1085.55**	9.14**	3.93**
Error	237	0.02	7222.89	0.09	1.56	15.18	1.34	12550.46	53.35	262.47	1.85	0.74
Total	319											

**-P<0.01; X1 = plant height (mt), X2 - root yield/plot (g), X3 = essential oil content (%), X4 = essential oil yield (g/plot), X5 = photosynthesis rate/net CO₂ assimilation rate (u mol m⁻² s⁻¹), X6 = transpiration rate (m mol m⁻² s⁻¹), X7 = stomatal conductance (m mol m⁻² s⁻¹), X8 = khusimol content (%), X9 = khusimol content (%), X10 = α -vetivone content (%), X11 = β - vetivone content (%).

Table 2: Vetiver genotypes included in the nine diverse clusters

Clusters	Numbers	Genotypes of vetiver												
		1	2	3	5	6	7	8	9	10	14	16	17	18
I st	56	19	21	23	24	26	27	28	30	31	32	33	35	36
		37	38	40	41	42	43	44	47	48	49	50	52	55
		56	57	58	59	63	64	65	67	68	69	72	73	74
		75	76	77	80									
2 nd	6	22	39	51	53	66	79							
3 rd	5	4	20	34	45	54								
4 th	3	12	29	46										
5 th	3	15	60	61										
6 th	2	11	13											
7 th	2	25	70											
8 th	2	62	71											
9 th	1	78												
9	80	n(n-1)/2 = 3160 pairs												

n = 80 genotype

Tables 3: Intra- and inter cluster D₂ values and distances (D) in the 80 genotypes of vetiver

Clusters	I st	2 nd	3 rd	4 th	5 th	6 th	7 th	8 th	9 th	\bar{D}^2
I st	19.72	29.52	32.34	40.20	43.13	31.99	42.02	31.73	39.85	34.50
D values	4.44	5.43	5.69	6.34	6.57	5.66	6.48	5.63	6.31	5.87
2 nd		23.69	46.19	50.07	51.72	38.84	46.64	37.80	32.81	39.70
D values		4.87	6.80	7.08	7.19	6.23	6.83	6.15	5.73	6.30
3 rd			29.08	37.32	62.34	45.56	85.52	71.37	62.87	52.51
D values			5.39	6.11	7.90	6.75	9.25	8.45	7.93	7.25
4 th				15.51	38.16	54.55	81.04	56.37	73.11	49.59
D values				3.94	6.18	7.39	9.00	7.51	8.55	7.04
5 th					19.72	49.78	43.03	56.98	44.92	45.53
D values					4.44	7.06	6.56	7.55	6.70	6.75
6 th						24.41	43.05	49.57	50.62	43.15
D values						4.94	6.56	7.04	7.11	6.57
7 th							23.81	40.15	46.92	45.46
D values							4.88	6.34	6.85	6.74
8 th								7.89	52.50	44.93
D values								2.81	7.25	6.70
9 th									0.00	44.84
D values									0.00	6.70

\bar{D}^2 - Average of inter cluster D² values; D values ($\sqrt{D^2}$) are in parenthesis; Intra cluster values in bold fonts

Table 4: The cluster mean of the eleven characters in vetiver genotypes

	X1	X2	X3	X4	X5	X6	X7	X8	X9	X10	X11
I st	1.56	427.30	0.87	3.34	7.19	4.56	282.30	15.30	19.87	2.87	2.60
2 nd	1.51	412.71	0.89	4.12	10.92	8.05	277.75	17.70	28.70	3.47	3.23
3 rd	1.54	414.00	0.96	3.01	10.47	4.80	224.30	23.39	43.53	5.61	1.84
4 th	1.54	546.25	0.90	2.11	13.25	4.43	711.58	18.09	14.39	3.56	2.17
5 th	1.64	502.08	1.48	6.25	11.88	4.54	676.58	22.72	46.60	1.36	2.80
6 th	1.51	435.63	0.98	0.75	7.11	4.44	296.63	23.59	5.80	4.38	4.18
7 th	1.56	441.88	0.83	8.94	10.73	5.04	267.75	16.65	36.91	1.71	1.96
8 th	1.49	487.50	0.61	3.65	11.28	3.87	258.75	1.95	34.87	0.66	0.66
9 th	1.69	502.08	2.09	4.25	11.53	4.27	263.00	16.63	39.90	3.43	2.27

X1 = plant height (mt), X2 - root yield/plot (g), X3 = essential oil content (%), X4 = essential oil yield (g/plot), X5 = photosynthesis rate/net CO₂ assimilation rate (u mol m⁻² s⁻¹), X6 = transpiration rate (m mol m⁻² s⁻¹), X7 = stomatal conductance (m mol m⁻² s⁻¹), X8 = khusimol content (%), X9 = khusimol content (%), X10 = α -vetivone content (%), X11 = β - vetivone content (%).

Table 5: Traits contributions (%), patterns at primary and secondary axis towards genetic diversity and other elite genetic parameters in vetiver

Characters	X1	X2	X3	X4	X5	X6	X7	X8	X9	X10	X11
Over all traits contributions (%)	2.69	3.29	10.73	11.20	3.30	13.32	13.99	7.44	5.60	9.46	18.99
Ranks	XI	X	V	IV	IX	III	II	VII	VIII	VI	I
Traits contributions (%) at primary axis	12.46	-64.39	79.82	183.09	-31.45	87.83	-153.71	-43.62	35.91	-91.69	85.76
Ranks at primary axis	VI	IX	IV	I	VII	II	XI	VIII	V	X	III
Traits contributions (%) at secondary axis	-0.0215	0.292	-0.299	0.194	-0.109	0.003	0.484	-0.170	0.320	-0.640	0.312
Ranks at secondary axis	VII	IV	X	V	IX	VI	I	VIII	II	XI	III
CD 5%	0.21	118.38	0.41	1.74	5.43	1.61	156.06	10.17	22.57	1.89	1.20
CD 1%	0.28	155.65	0.55	2.28	7.14	2.12	205.17	13.38	29.67	2.49	1.58
CV (%)	9.87	19.61	32.68	35.30	46.85	24.03	36.33	44.84	68.92	45.06	33.57

X1 = plant height (mt), X2 - root yield/plot (g), X3 = essential oil content (%), X4 = essential oil yield (g/plot), X5 = photosynthesis rate/net CO₂ assimilation rate (u mol m⁻² s⁻¹), X6 = transpiration rate (m mol m⁻² s⁻¹), X7 = stomatal conductance (m mol m⁻² s⁻¹). X8 = khusimol content (%), X9 = khusinol content (%), X10 = α -vetivone content (%), X11 = β -vetivone content (%).

Table 6: Mean performance of the six selected high essential oil yielding genotypes of vetiver

Genotypes	X1	X2	X3	X4	X5	X6	X7	X8	X9	X10	X11
70	1.58	472.50	0.83	9.25	14.43	3.35	307.25	22.04	55.61	1.52	2.85
25	1.54	411.25	0.83	8.63	7.03	6.73	228.25	11.25	18.21	1.90	1.07
60	1.81	485.00	1.63	7.75	11.53	4.53	789.75	24.03	44.48	1.75	2.63
15	1.77	496.25	1.55	6.00	10.54	4.39	717.50	28.05	39.80	1.17	3.55
6	1.42	522.50	0.81	6.00	4.00	4.27	249.00	6.15	10.78	1.16	1.09
3	1.49	375.00	0.88	5.50	7.70	4.14	215.50	7.20	43.01	2.14	2.21

X1 = plant height (mt), X2 - root yield/plot (g), X3 = essential oil content (%), X4 = essential oil yield (g/plot), X5 = photosynthesis rate/net CO₂ assimilation rate (u mol m⁻² s⁻¹), X6 = transpiration rate (m mol m⁻² s⁻¹), X7 = stomatal conductance (m mol m⁻² s⁻¹). X8 = khusimol content (%), X9 = khusinol content (%), X10 = α -vetivone content (%), X11 = β -vetivone content (%).

Supplementary Table 1: Origin of eighty genotypes of vetiver

Genotypes	Codes	Origin	Genotypes	Codes	Origin
1	MBR-1	Raza Ganj Lakhim Pur (Kheri) -U.P. North India	41	KH-41	Kanpur U.P., (North India)
2	MBR-2	Raza Ganj Lakhim Pur (Kheri) -U.P. North India	42	KH-3	Kanpur U.P., (North India)
3	MBR-3	Raza Ganj Lakhim Pur (Kheri) -U.P. North India	43	KH-2	Kanpur U.P., (North India)
4	TRAV-3	Travancore - Kerala (South India)	44	KH-2	Kanpur U.P., (North India)
5	MBR-5	Bharatpur, Rajasthan (North India)	45	K.S-2	CSIR-CIMAP, Lucknow U.P., (North India)
6	MBR-6	Razaganj Lakhim Pur (Kheri)-U.P. (North India)	46	MTR-1	Mathura, U.P., (North India)
7	MBR-7	Razaganj Lakhim Pur (Kheri)-U.P. (North India)	47	HD-2	Hardoi U.P., (North India)
8	GHT-1	Ghaghra Ghat, U.P. (North India)	48	PUR-1	Purara (Almora) Uttrakhand, (North India)
9	MBJ-1	Jaiti Pur (Unnao), U.P. (North India)	49	PUR-2	Purara (Almora) Uttrakhand, (North India)
10	MBJ-2	Jaiti Pur (Unnao), U.P. (North India)	50	PUR-3	Purara (Almora) Uttrakhand, (North India)
11	MBJ-3	Jaiti Pur (Unnao), U.P. (North India)	51	BDP-2	Pant Nagar Uttrakhand, (North India)
12	HARG-1	Hargaon (Sita Pur), U.P. (North India)	52	BDP-3	Pant Nagar Uttrakhand, (North India)
13	KASM-2	Kasmanda (Sita Pur), U.P. (North India)	53	BDP-4	Pant Nagar Uttrakhand, (North India)
14	SIDH-3	Sidhuli (Sita Pur), U.P. (North India)	54	BDP-5	Pant Nagar Uttrakhand, (North India)
15	MAST-4	Masterbag (Sita Pur), U.P. (North India)	55	BDP-6	Pant Nagar Uttrakhand, (North India)
16	BDP-1	Pantnagar (U.S.Nagar), Uttrakhand (North India)	56	BDP-7	Pant Nagar Uttrakhand, (North India)
17	KS-1	CSIR-CIMAP, Lucknow, U.P., (North India)	57	BDP-8	Pant Nagar Uttrakhand, (North India)
18	Sugandha	CSIR-CIMAP, Lucknow, U.P., (North India)	58	BDP-9	Pant Nagar Uttrakhand, (North India)
19	Pusa Hyb-28	New Delhi North India	59	MZFP-1	Muzaffer Pur – Katra, Bihar, (North India)
20	Pusa Hyb-8	New Delhi North India	60	MZFP-2	Muzaffer Pur – Katra, Bihar, (North India)
21	Pusa Hyb-7	New Delhi North India	61.	MZFP-3	Pahlaja Ghat Bihar, (North India)
22	R.I. - 1	Reunion	62.	MZFP-4	Baniyawan Bihar, (North India)
23	TRAV-4	Travancore - Kerala (South India)	63.	MZFP-5	Sahu Pokhar Bara Mandir-Bihar (North India)
24	OD-1	LRC, Odakali (South India)	64.	GHS-1	Ghana Sanctuary (South Africa)
25	BL-1	Bareilly-U.P., (North India)	65.	GHS-2	Ghana Sanctuary (South Africa)
26	BMM-1	Maholi (Sita Pur) U.P., (North India)	66.	GHS-3	Ghana Sanctuary (South Africa)
27	BB-1	Baheri (Bareilly) Uttrakhand (North India)	67.	GHS-4	Ghana Sanctuary (South Africa)
28	I.N-1	Indonesia	68.	GHS-6	Ghana Sanctuary (South Africa)
29	B.J-1	Baba Ganj U.P., (North India)	69.	RJ-1	Jammu (J. & K.) North India
30	GHT-2	Ghaghra Ghat U.P., (North India)	70.	RJ-2	Jammu (J. & K.) North India
31	BST-1	Basari Ghat U.P., (North India)	71.	RJ-3	Jammu (J. & K.) North India
32	BKT-1	Baksi-Ka-Talab (Lucknow) U.P., (North India)	72.	PUN-1	Phagwara, Punjab (North India)
33	KH-55	Kanpur U.P., (North India)	73.	PUN-2	Jalandhar, Punjab (North India)
34	KH-26	Kanpur U.P., (North India)	74.	PUN-3	Ludhiana, Punjab (North India)
35	KH-23	Kanpur U.P., (North India)	75.	PUN-4	Amritsar, Punjab (North India)
36	KH-16	Kanpur U.P., (North India)	76.	STR-1	Trichur, Karala (South India)
37	KH-11	Kanpur U.P., (North India)	77.	STR-2	Trichur, Karala (South India)
38	KH-8	Kanpur U.P., (North India)	78.	CTK-1	uttack, Odisha (India)
39.	KH-30	Kanpur U.P., (North India)	79.	CTK-1	Cuttack, Odisha (India)
40.	KH-40	Kanpur U.P., (North India)	80.	SHG-1	Shakchhi Gopal, Odisha (india)

Supplementary Table 2: Mean performance of the 80 vetiver genotypes

Genotypes	X1	X2	X3	X4	X5	X6	X7	X8	X9	X10	X11
1	1.49	435.00	1.21	2.83	4.89	4.04	257.25	10.13	3.87	1.93	2.65
2	1.61	575.00	1.66	3.50	7.93	4.38	267.75	7.25	0.09	2.82	3.06
3	1.49	375.00	0.88	5.50	7.70	4.14	215.50	7.20	43.01	2.14	2.21
4	1.42	412.50	0.93	0.67	8.60	4.55	230.00	25.28	44.64	6.76	1.21
5	1.71	437.50	0.94	2.80	4.33	3.97	313.25	9.75	9.03	2.92	1.33
6	1.42	522.50	0.81	6.00	4.00	4.27	249.00	6.15	10.78	1.16	1.09
7	1.74	475.00	1.50	4.25	11.05	4.91	265.50	5.43	11.95	2.96	1.52
8	1.62	437.50	0.63	2.88	11.93	4.02	270.50	25.35	4.28	2.91	2.27
9	1.35	331.25	0.80	3.68	11.21	4.61	199.25	20.38	43.69	4.90	3.52
10	1.64	417.25	2.09	2.58	4.75	3.82	297.25	18.31	2.66	2.17	3.75
11	1.69	348.75	0.88	0.83	3.03	3.97	203.25	32.88	2.58	3.26	4.45
12	1.61	508.75	0.84	3.33	16.93	4.27	713.75	19.62	12.18	4.38	3.79
13	1.32	522.50	1.08	0.67	11.20	4.91	390.00	14.30	9.03	5.50	3.91
14	1.53	475.00	0.76	2.80	7.53	3.82	499.00	16.15	9.03	1.29	3.98
15	1.77	496.25	1.55	6.00	10.54	4.39	717.50	28.05	39.80	1.17	3.55
16	1.60	475.00	0.66	4.25	6.70	4.22	540.50	9.06	44.68	2.08	3.65
17	1.73	437.50	0.56	2.88	8.60	4.66	281.75	22.55	28.88	3.39	3.31
18	1.70	400.00	0.64	3.68	8.60	4.61	267.75	11.30	5.50	1.85	3.30
19	1.52	460.00	0.59	2.75	5.15	3.82	256.25	33.68	9.30	3.05	3.58
20	1.58	575.00	0.66	3.00	4.30	3.97	198.50	33.55	33.93	5.74	1.17
21	1.80	375.00	0.56	2.75	6.78	4.27	285.75	17.65	13.91	2.22	2.66
22	1.46	407.50	0.68	3.63	14.43	8.76	292.00	14.25	2.69	2.70	3.66
23	1.56	450.00	1.31	2.38	6.23	4.94	241.25	20.80	9.95	3.44	4.18
24	1.54	560.00	0.80	2.13	5.98	7.29	287.00	8.25	4.70	1.80	2.15
25	1.54	411.25	0.83	8.63	7.03	6.73	228.25	11.25	18.21	1.90	1.07
26	1.47	463.75	0.61	3.43	3.63	4.82	266.75	19.73	29.20	3.23	1.35
27	1.49	362.50	0.54	3.55	11.53	3.68	196.75	6.20	11.90	2.58	1.26
28	1.53	452.50	0.64	1.83	13.58	3.30	410.25	11.98	18.60	3.75	1.20
29	1.51	505.00	0.74	1.30	10.58	4.37	658.75	17.85	26.23	2.32	1.34
30	1.43	412.50	0.70	0.50	9.43	4.23	261.50	12.75	12.65	5.49	3.03
31	1.58	450.00	0.78	3.50	5.15	4.53	201.00	28.58	18.50	2.99	4.18
32	1.47	302.50	0.75	5.13	4.30	4.70	312.75	12.63	27.95	2.90	3.60
33	1.54	327.50	2.06	3.98	6.78	3.93	270.50	6.63	8.13	1.77	3.33
34	1.58	287.50	0.86	3.63	14.43	4.55	248.25	26.50	41.73	6.34	3.43
35	1.51	390.00	0.79	4.55	11.99	3.47	288.25	16.60	9.03	4.94	3.30
36	1.52	568.75	0.90	3.75	6.80	7.14	272.00	5.38	8.13	4.34	2.81
37	1.40	385.00	0.76	4.13	10.15	7.01	196.50	27.95	14.20	1.51	3.53
38	1.58	403.00	1.25	5.43	7.00	4.89	257.75	21.50	15.13	3.10	2.36
39	1.47	373.75	0.78	4.75	5.98	7.72	314.00	33.45	41.78	5.68	3.68
40	1.45	302.75	0.93	4.43	8.78	6.48	233.25	10.70	14.08	2.41	2.38
41	1.51	370.00	1.13	4.00	4.15	3.61	277.00	16.00	28.70	2.14	2.84
42	1.51	415.00	0.82	3.38	9.78	3.35	257.00	15.38	52.63	3.61	2.80
43	1.54	296.00	0.87	3.00	2.90	3.82	225.25	19.30	7.52	2.57	1.45
44	1.53	397.50	0.86	3.80	9.80	4.39	247.00	15.60	14.00	3.24	2.88
45	1.51	282.50	1.50	4.73	12.50	4.22	230.75	17.83	45.50	5.61	1.64
46	1.52	625.00	1.11	1.72	12.25	4.66	762.25	16.80	4.77	4.00	1.39
47	1.64	473.75	0.63	2.25	9.98	4.61	311.50	14.68	5.88	4.83	2.40
48	1.67	400.00	0.61	3.63	6.00	3.82	235.75	29.38	41.40	4.75	3.73
49	1.47	475.00	0.98	3.06	4.00	3.97	201.00	18.11	11.56	6.38	2.60
50	1.76	497.50	0.76	3.48	3.03	4.27	312.75	32.55	12.30	3.89	1.24
51	1.49	436.25	1.01	3.63	14.95	8.76	270.50	18.96	13.68	6.71	3.33
52	1.40	540.00	0.79	4.38	2.90	4.94	248.25	12.02	50.25	1.09	3.43
53	1.70	462.50	1.42	4.70	9.80	7.29	283.75	8.95	50.93	1.17	3.85
54	1.63	512.50	0.84	3.05	12.50	6.73	214.00	13.78	51.88	3.58	1.76
55	1.44	425.00	0.74	3.00	12.25	4.82	307.25	8.15	6.25	2.18	1.14
56	1.46	560.00	0.85	1.48	6.10	3.68	174.50	9.58	31.66	2.05	1.85
57	1.72	411.25	0.85	4.00	5.98	3.30	562.00	12.03	51.38	1.07	3.29
58	1.54	438.75	0.84	0.88	7.03	4.37	556.25	10.24	17.58	3.90	1.95
59	1.55	425.00	0.96	1.88	3.63	4.23	350.75	12.07	26.58	1.57	2.09
60	1.81	485.00	1.63	7.75	11.53	4.53	789.75	24.03	44.48	1.75	2.63
61	1.33	525.00	1.26	5.00	13.58	4.70	522.50	16.09	55.52	1.17	2.23
62	1.48	400.00	0.58	4.30	10.58	3.93	343.00	2.56	28.29	0.53	0.53
63	1.79	331.25	0.65	4.25	9.43	4.55	235.75	11.15	40.13	2.54	1.51
64	1.49	454.75	0.60	3.38	5.15	3.47	201.00	9.68	17.13	2.67	1.13
65	1.47	336.25	0.71	3.50	4.30	7.14	312.75	9.15	20.28	2.34	1.44
66	1.48	508.75	0.56	3.63	6.78	7.01	270.50	11.55	52.63	1.09	3.08
67	1.72	485.00	0.76	3.88	11.38	4.89	248.25	13.35	18.58	3.39	1.77

68	1.81	525.00	1.36	2.75	4.30	7.72	283.75	6.04	36.60	2.33	3.13
69	1.54	400.00	0.84	2.08	6.78	6.06	214.00	24.10	31.58	1.12	1.76
70	1.58	472.50	0.83	9.25	14.43	3.35	307.25	22.04	55.61	1.52	2.85
71	1.51	575.00	0.64	3.00	11.99	3.82	174.50	1.34	41.45	0.80	0.79
72	1.45	375.00	0.56	3.55	6.80	4.39	410.75	9.13	10.50	3.71	3.04
73	1.46	425.00	0.69	3.20	10.15	4.22	272.00	22.95	14.83	1.84	3.44
74	1.62	427.50	0.76	1.73	7.00	4.66	196.50	25.75	14.63	3.40	2.00
75	1.47	442.50	0.70	1.13	5.98	4.61	257.75	28.13	42.53	5.40	3.88
76	1.41	450.00	0.78	4.50	7.03	3.82	314.00	13.15	10.03	2.36	2.28
77	1.70	302.50	0.80	5.00	3.63	3.97	233.25	14.88	41.63	2.14	3.11
78	1.69	327.50	2.09	4.25	11.53	4.27	263.00	16.63	39.90	3.43	2.27
79	1.46	287.50	0.88	4.38	13.58	8.76	235.75	19.05	10.50	3.50	1.80
80	1.54	390.00	0.84	4.00	10.58	4.94	201.00	14.10	13.75	2.21	3.11

X1 = plant height (mt), X2 = root yield/plot (g), X3 = essential oil content (%), X4 = essential oil yield (g/plot), X5 = photosynthesis rate/net CO₂ assimilation rate (u mol m⁻² s⁻¹), X6 = transpiration rate (m mol m⁻² s⁻¹), X7 = stomatal conductance (m mol m⁻² s⁻¹). X8 = khusimol content (%), X9 = khusinol content (%), X10 = α-vetivone content (%), X11 = β- vetivone content (%).

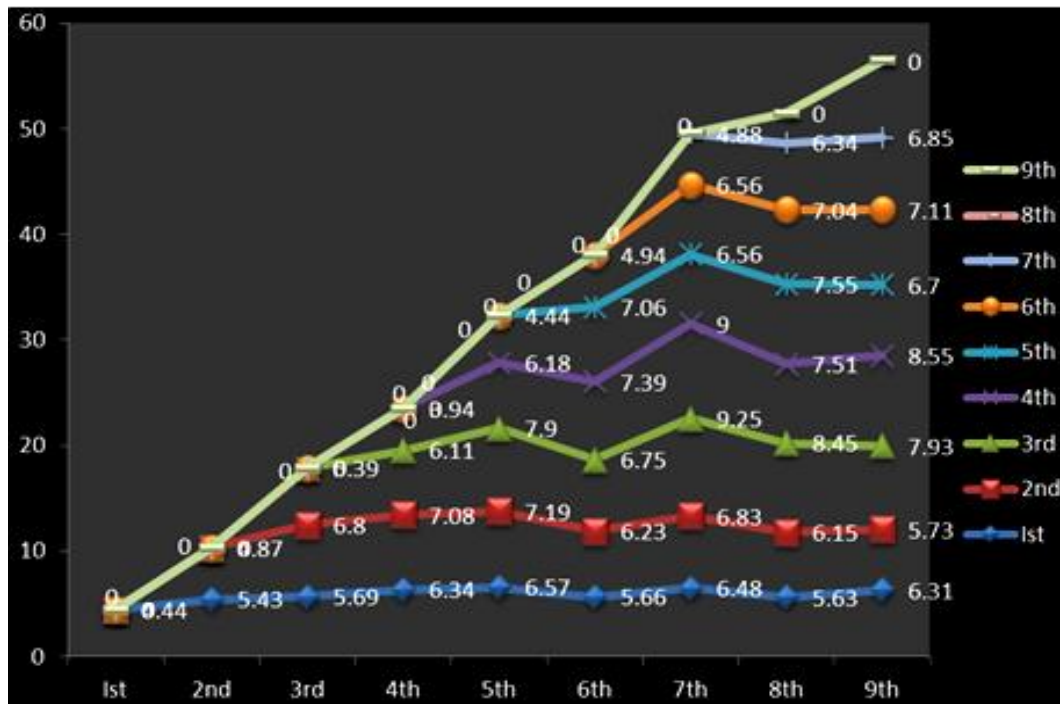


Fig 2: Intra-inter distance among the nine diverse clusters in the vetiver genotypes

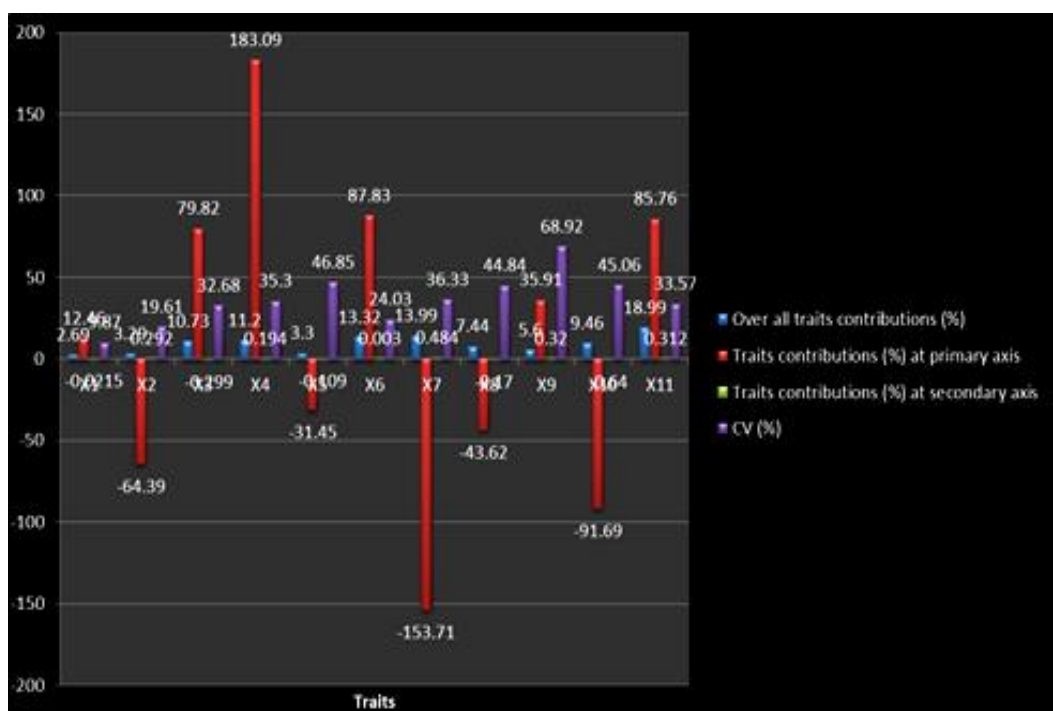


Fig 3: Character contributions (%), patterns at primary and secondary axis towards genetic diversity in the 80 genotypes of vetiver

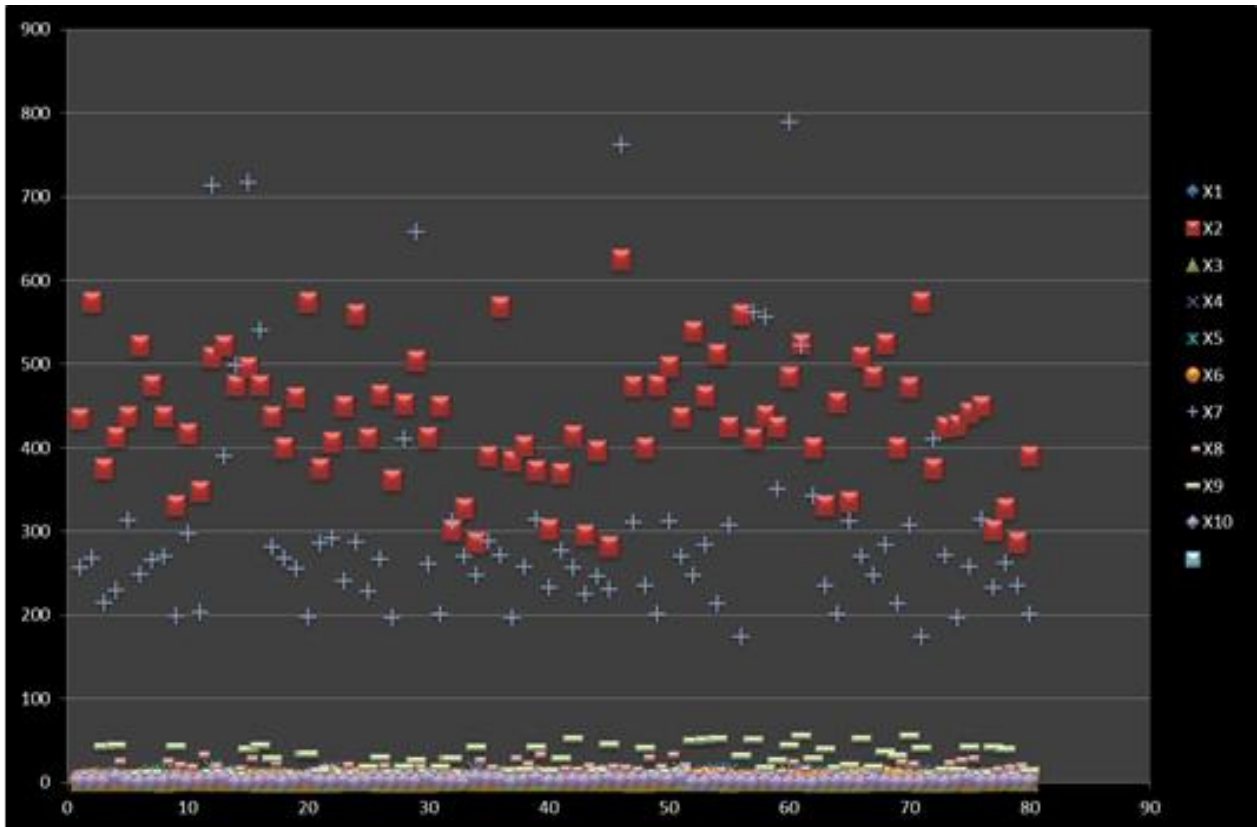


Fig 4: Traits wise distributions of the 80 vetiver genotypes

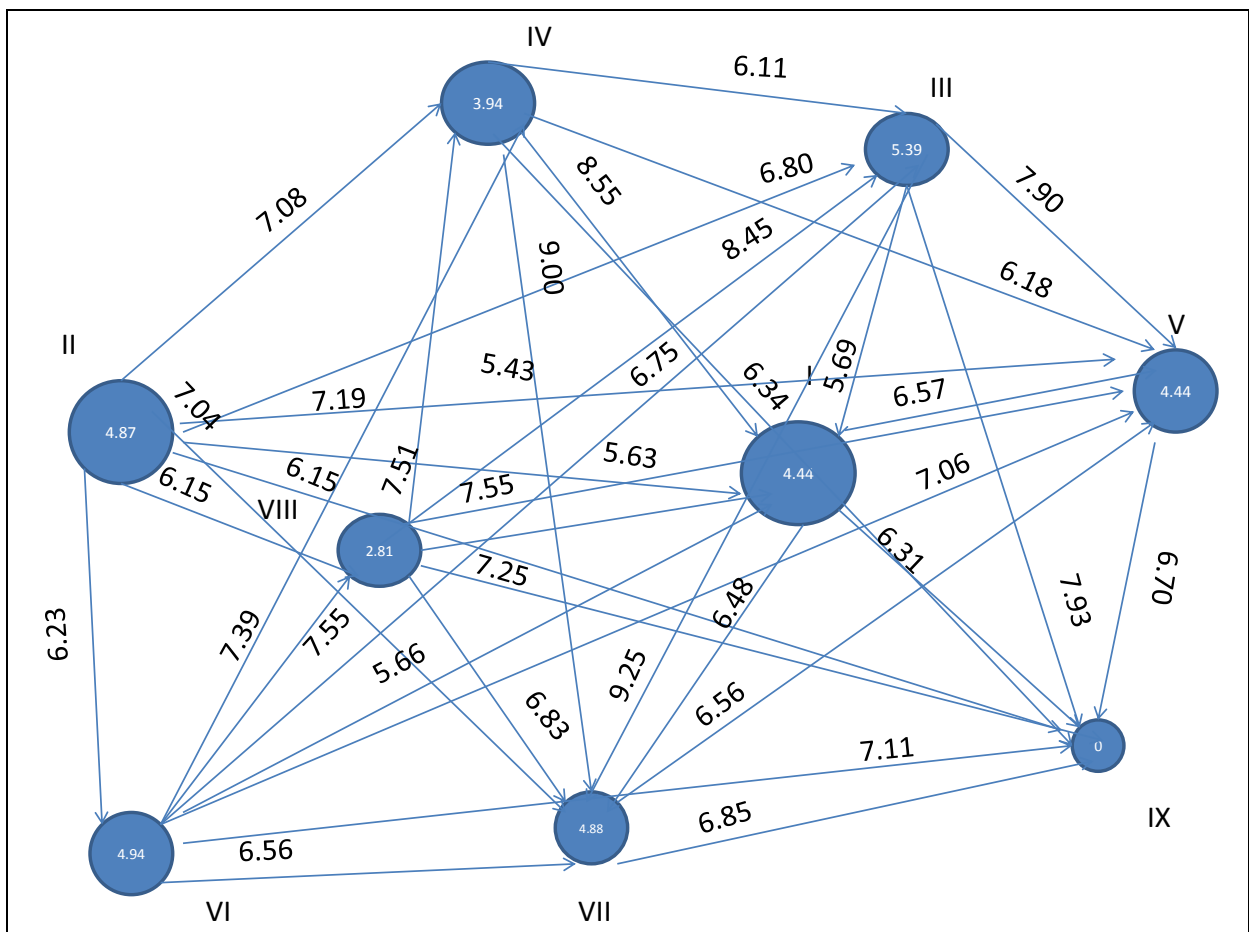


Fig 5: Distances (D) between the nine diverse clusters of vetiver

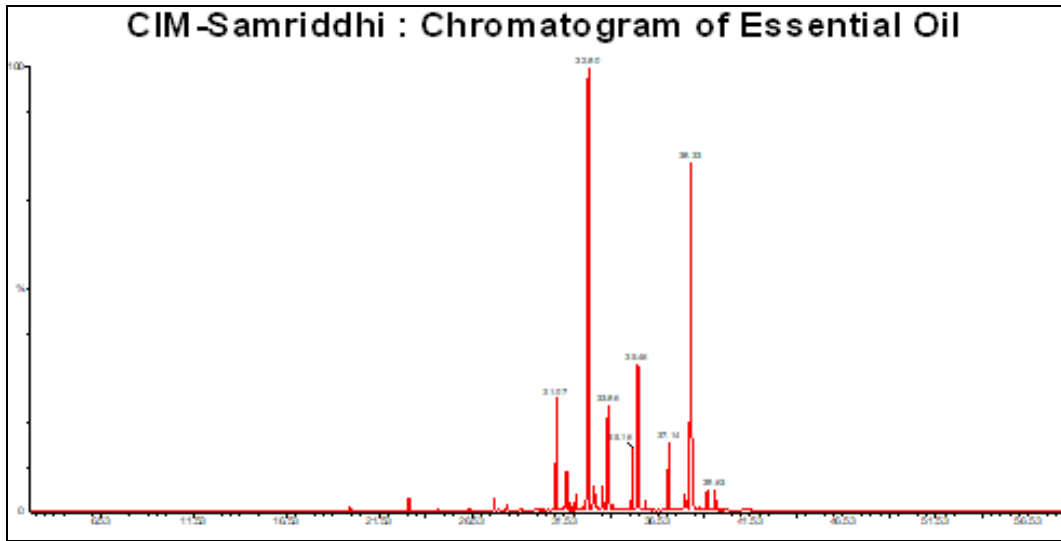


Fig 6: Chromatogram of the essential oil of No. 70 (CIM Samridhi)

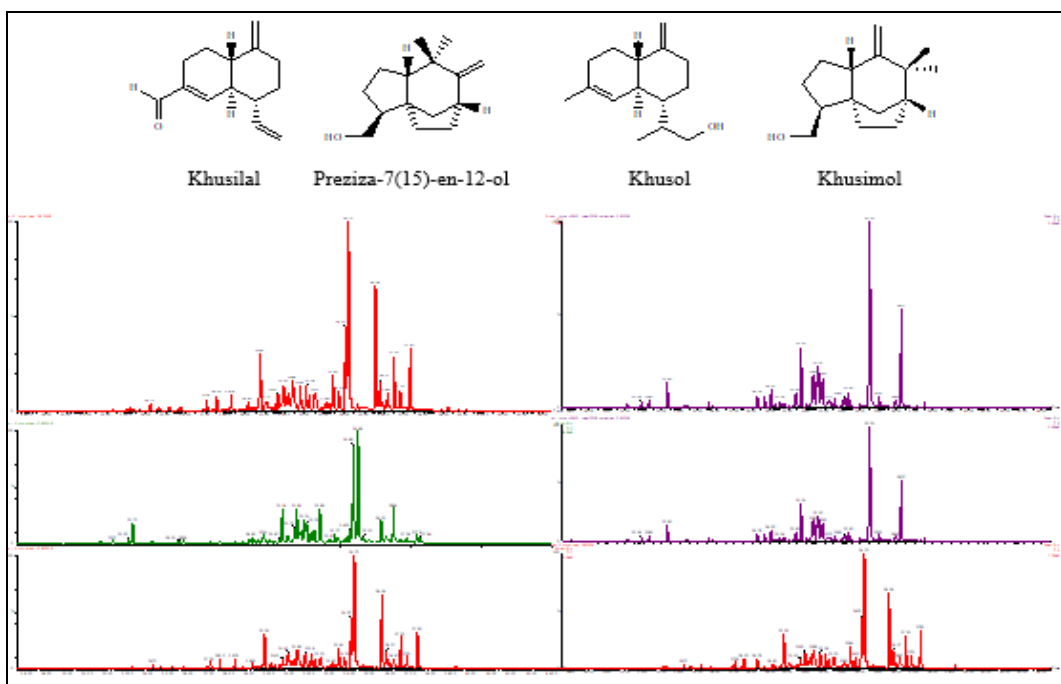


Fig 7: Chromatogram of the essential oil of some selected genotypes and chemical structures of some chemical compounds of vetiver

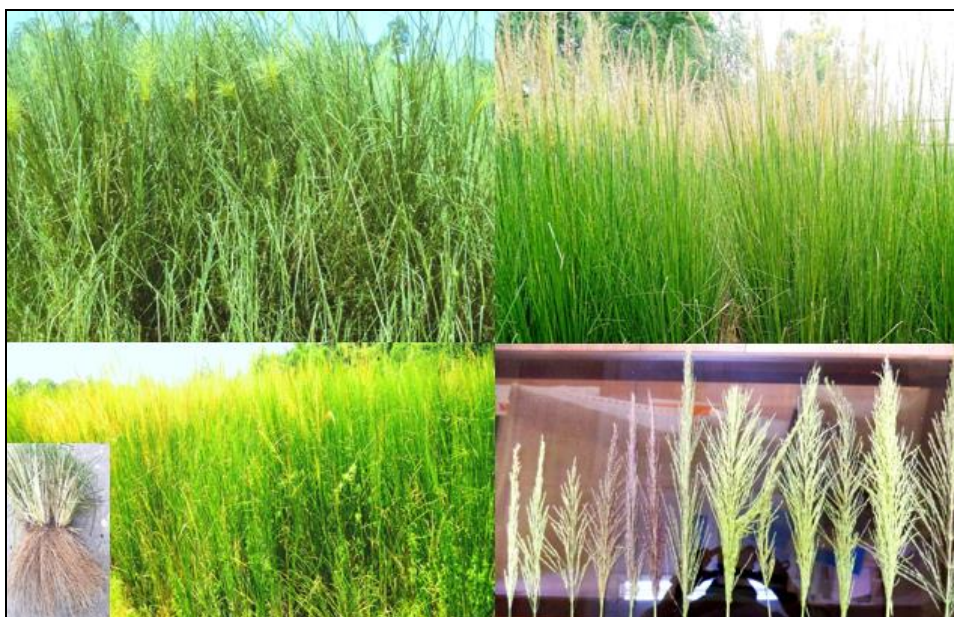


Fig 8: Drooping vs erect leaves, colour variations in the inflorescence, and variations in the roots of vetiver genotypes

4. Conclusions

Multivariate analysis was utilized to determine the genetic diversity of 80 vetiver accessions using eleven of the most commercially important traits. To better understand the genetic variation character contribution of key yield components in vetiver, the accessions/genotypes were classified into nine distinct clusters. The genotypes No. 70, 25, 60, 6 and 15 exhibited a lot of genetic divergence. Therefore selecting these accessions could lead to a lot of genetic gains in terms of essential oil yield traits. Traits like X10, X4, X5, X6, and X11 were the major contributors to genetic divergence. These selections as Nos. 70, 25, 60, 15, 6, and 3 can be utilized to make commercial vetiver.

5. Acknowledgement

The first author is grateful to the CSIR for providing financial support for the research described above activity through the Emeritus Scientist scheme No. 21(1020)/16/EMR-II dated November 18, 2016. The authors are also appreciative to the Director, CSIR-CIMAP, Lucknow, Uttar Pradesh, India, PIN 226 015, for providing the essential facilities and infrastructure for the research. CIMAP Communication Number: CIMAP/PUB/2021/MAR/17.

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