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Research Article

Genotypic and Morphological Appearance of the Traits in Relation to Genetic Diversity of Essential Oil Yield in Vetiver Grass (*Chrysopogon zizanioides* Roberty)

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Abstract

The nature and magnitude of genetic divergence were estimated in 60 vetiver (*Chrysopogon zizanioides*) genotypes using Mahalanobis D^2 -Statistics by considering four most economic traits. Mahalanobis D^2 analysis revealed considerable amount of diversity in the vetiver. The genotypes were grouped into six clusters. Cluster I constituted maximum number of genotypes (36) followed by cluster II (14); III (6), IV (2) and one each in the cluster V and VI, respectively. The genotypes falling under cluster VI had the maximum divergence (163.20), followed by cluster III (118.13) and cluster II (67.52). The highest inter-cluster distances were observed between cluster III and VI (372.10) cluster II and cluster VI (235.77) and Cluster I and VI (207.35), suggesting that the genotypes constituted in these clusters may be used for future breeding programme. Traits like essential oil yield, root yield and oil content was the major contributors to genetic divergence. Accessions No. 30 (DH-1) followed by No. 12 (CIM Samriddhi, No. 42 (Selection -1), No. 36 (Gulabi) and No. 60 (CIM Vriddhi) can be exploited for commercial cultivation.

Keywords: Clusters; Diversity; Distillation; Genetic Variability; Heritability; Vetiver Grass

Introduction

The vetiver (*Vetiveria zizanioides* (L.) Nash syn. *Chrysopogon zizanioides* Roberty, family-Poaceae, is native to India and is found growing wild in almost all parts of the country. Roots of vetiver are the source of world-renowned 'khus' oil that has considerable value in essential oil industries [1,2]. It can be grown on marginal soils, including saline and sodic, sandy, waterlogged and sloppy land. Out of the two species occurring in India, *Vetiveria zizanioides* and *Vetiveria lawsonii* syn. *V. nemoralsis*, only the former has commercial significance because of high class perfumery value of its oil, known since ancient times [3-5]. In India, vetiver grows luxuriantly in wild in Uttar Pradesh, Bihar, Rajasthan (northern states), Southern and peninsular India and its cultivation is also done on limited scale in Andhra Pradesh, Tamilnadu and Kerala [5-8]. Quality of vetiver oil, especially from north India, is considered to be the best in the world. The major vetiver oil producing countries are Haiti, Indonesia and India. Vetiver is also grown in over 100 countries for its environmental application for soil/water conservation and carbon sequestration. The total world production of vetiver oil is estimated to be 300 - 350 tons per year. The annual consumption and demand is likely to increase further. In India, about 100 tons of oil is produced annually, which is far below to meet our internal demand of the oil for perfumery, masticatory, attar and soap industries. Recently, farmers in north India have taken up vetiver cultivation on large scale due to high demand of essential oil of vetiver.

The natural genetic variability in this crop is very high. Since genetic variability for diverse most economic traits in the vetiver genetic accessions is a prelude to potential crop improvement, the CSIR-Central Institute of Medicinal and Aromatic Plants, Lucknow is maintaining 60, indigenous and exotic collections of vetiver, representative of twelve states of India (Uttar Pradesh, Uttaranchal, Rajasthan, Bihar, Punjab, Madhya Pradesh, Gujarat, Jammu and Kashmir, Odisha, Maharashtra, Kerala, Andhra Pradesh) and four exotic collections from Indonesia, Haiti, Thailand and Reunion Island. CSIR-CIMAP also developed and released ten essential oil high yielding varieties of vetiver of different notes in essential oil namely, KS-1, Sugandha, KS-2, Dharini, Gulabi, Kesari, CIM Vriddhi, CIMAP-KHUS 40 (induced tetraploids), G22, G15, CIM-Khusinolika and CIM Samriddhi for commercial cultivation. By utilizing the genetic diversity in this crop, there are further possibility to development of high root and better quality essential oil yielding varieties. The khus cultivation in Indian context seems to be profit-driven with increasing oil demand for perfume and soap industries. Khus cultivation gives a net profit of over Rs 1.5 lacs/ha in a span of 10 - 12 months with 25 - 30 kg/ha oil yield. In addition, co-cultivation of khus with wheat, paddy, lentil, peas, mint, basil etc. brings an additional profit of about Rs. 30,000/ha.

Looking at the large quantities of Vetiver oil still imported, there is a need to further enhance the area under khus cultivation, by popularizing high oil yielding and early maturing varieties of this crop. Due to its economic importance and availability of the genetic diversity, efforts have been made to collect and characterize the germplasm collection. The present investigation has been undertaken to assess the genetic divergence among the genotypes and selection of desirable accessions/genotypes for further exploitation in vetiver crop.

Materials and Methods

Research material: Sixty vetiver accessions/clones from indigenous and exotic collections of vetiver (*Vetiveria zizanioides* (L.) Nash ex Small syn. *Chrysopogon zizanioides* Roberty), representative of twelve states of India (Uttar Pradesh, Uttaranchal, Rajasthan, Bihar, Punjab, Madhya Pradesh, Gujarat, Jammu and Kashmir, Odessa, Maharashtra, Kerala and Andhra Pradesh) and four exotic collections from Indonesia, Haiti, Thailand and Reunion Island constituted the materials for the present study (Table 1). They were evaluated at the Research Farm of CSIR-Central Institute of Medicinal and Aromatic Plants, P.O. CIMAP, Lucknow, U.P. 226 015 (India) in the two consecutive growing seasons/years: 2014 - 2015 and 2015 - 2016 of Northern Indian plain's environment in a randomized complete block design with three replications. The slips were planted in 50 × 50 cm rows to row/plants to plant. The plants given the normal intercultural operations, irrigation, and fertilizer applications (100 kg N, 80 kg P2O5, and 40 kg K2O per hectare). Plants were harvested by uprooting 12 months after planting. Essential oil was extracted from the roots by hydro-distillation using Clevenger type apparatus [9].

Observations recorded: Morpho-metric observations were recorded for four most economic traits, namely plant height (cm), root yield (g/plot), oil content (%) and oil yield (g/plot) for D^2-statistics and other allied genetic parameters.

Statistical analysis: Data were analyzed using statistical software version 0.3 available at CSIR–CIMAP, Lucknow India in the department of Genetics and Plant Breeding for genetic diversity following D^2- statistics [10,11]. D^2- analysis was used for assessing the genetic divergence among the test entries involving quantitative characters. All n(n-1)/2 D^2 values was clustered using Tocher's method described by Rao [12] based on Singh and Chaudhary [13].

Results and Discussion

The analysis of variance for different traits revealed highly significant differences among the treatments, accessions/genotypes, years and genotypes × Year for the all four characters, clearly indicated the high degree of genetic variability were present in the material evaluated and existence of considerable genetic diversity among genotypes. The sixty accessions/genotypes grouped into six different clusters (cluster I, II, III, IV, V and VI (Table 1-3). Among the clusters, the maximum number of accessions (36) were included in Cluster I followed by cluster II (14), cluster III (6) and cluster IV (2). Clusters V and VI accommodated one accession/genotypes each. The highest inter cluster distance was observed between cluster III and VI (372.10) and minimum inter cluster distance between cluster I and II (16.25) (Table 3). Highest average ($\bar{D}^2$) values was recorded in cluster VI (163.20) followed by cluster III (118.13), Cluster II (67.52), cluster I (63.08), Cluster IV (61.65) and cluster V (61.27) in the study (Table 3). The cluster IV showed highest mean value for essential oil content (1.23%) and essential oil yield (4.69 g/plot) followed by cluster I essential oil content (0.95%) and essential oil yield (3.70 g/plot). cluster V recorded the tallest plant (Table 4; Figure 1-4). Desirable types can be selected from the clusters based on the objective of the breeding programmes. Generally, geographical diversity has been considered as a measure of genetic diversity. However, this is an inferential criterion and it may not be so effective in quantifying different populations.

The present pattern of grouping of genotypes indicated that the genetic diversity was not fully related to the geographical diversity. Absence of relationship between the genetic diversity and geographical distance was thus brought to focus by the present study. These findings are in confirmation of results obtained by Virmani and Datta [3], Pareek [2], Husain., *et al*. [1], Lal., *et al*. [6], Lal [14] and Lal., *et al*. [5] in the vetiver. Genetically diverse types accessions having useful characters from the same or different region can be effectively used for subsequent breeding programmes.

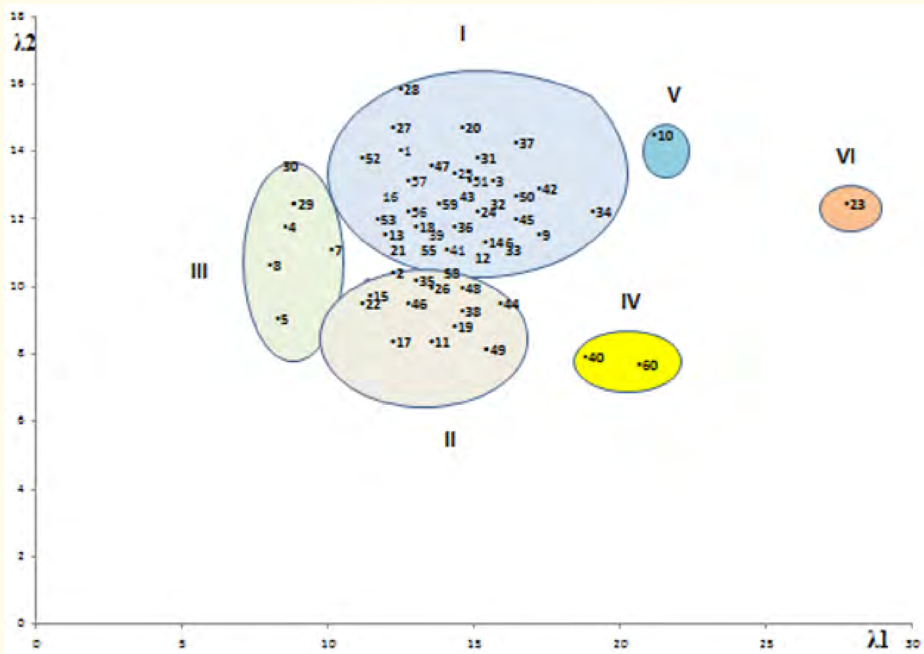

Figure 1: λ 1-λ2 chart for the distribution pattern of 60 accessions of vetiver.

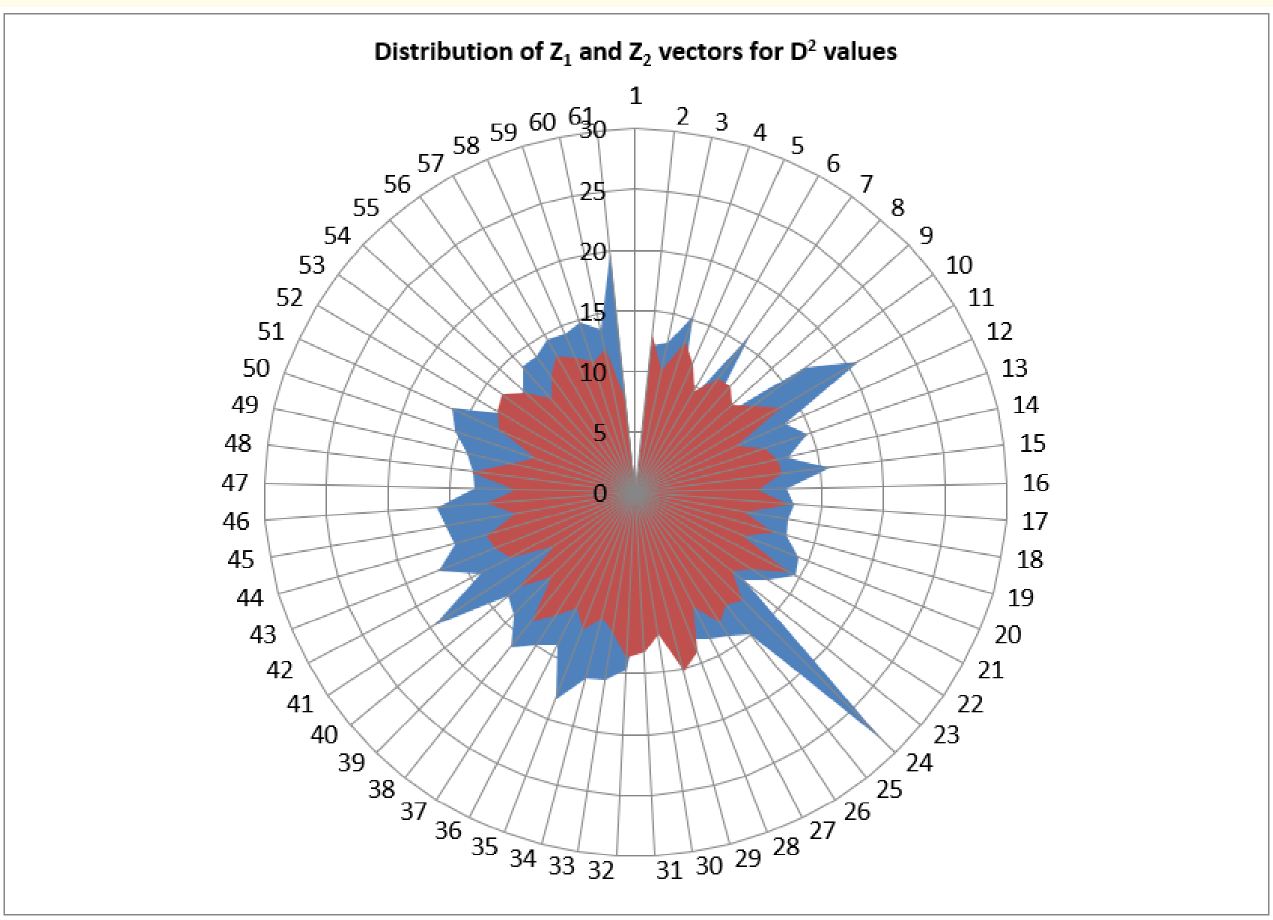

Figure 2: Distribution of Z1 (blue colour) and Z2 (Red colour) vector for D2 values of 60 accessions of vetiver.

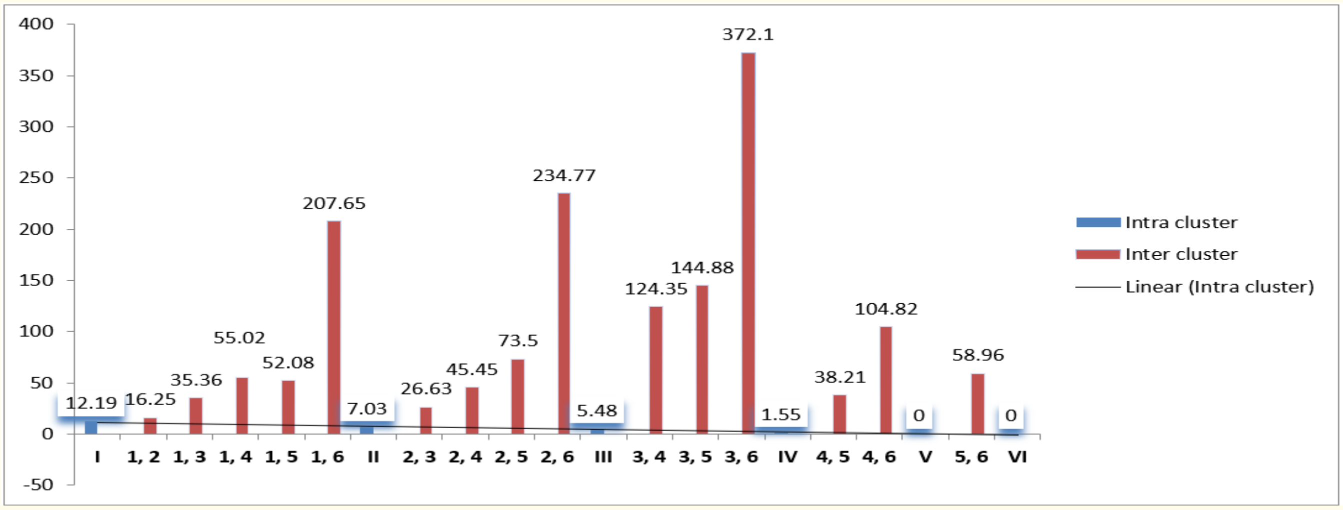

Figure 3: Intra-and inter cluster distances between 60 accessions of vetiver.

Figure 4: Variations in the root length of one year crop of vetiver accessions.

S. No.	Accession's code	Appearance	Origin
1.	Veti-1	Medium tall	Muzafferpur (Bihar) (India)
2.	Veti-2	Medium tall	Ghana Sanctuary, Bharat Pur, Rajasthan (India)
3.	Veti-3	Medium tall	Jannu Tawi, Jammu and Kashmir
4.	Veti-4	Medium tall	Phagwara, Punjab (India)
5.	Veti- 5	2nd tallest	Cuttak, Orissa (India)
6.	Veti- 6	Dwarfest	Bhubeneswer, Orissa (India)
7.	Veti- 7	Tall	Thailand
8.	Veti- 8	Medium tall	Haiti
9.	Veti- 9	Medium tall	Nimach, M.P. (India)
10.	Veti- 10	Tall	CIMAP, Lucknow, (India)

11.	Veti- 11	Medium tall	CIMAP, Lucknow, (India)
12.	Veti -12 (Var. CIM-Samriddhi)	Medium tall	CIMAP, Lucknow, (India)
13.	Veti- 13	Medium tall	Baba Ganj, U.P. (India)
14.	Veti- 14	Tall	Mathura, U.P. (India)
15.	Veti- 15	Medium tall	Pantnagar, Uttrakhand (India)
16.	Veti-16	Medium tall	Muzaffarpur (Bihar) (India)
17.	Veti-17	Medium tall	Ghana Sanctuary, Bharat Pur, Rajasthan (India)
18.	Veti-18	Medium tall	Jannu Tawi, Jammu and Kashmir
19.	Veti-19	Medium tall	Phagwara, Punjab (India)
20.	Veti-20 (Var. Dharini)	Very Tall	CIMAP, Lucknow, (India)
21.	Veti- 21	Medium tall	Bhubeneswer, Orissa (India)
22.	Veti-22	Medium tall	Agra, U.P., (North India)
23.	Veti-23	Tall	Musanagar, U.P. (India)
24.	Veti-24	Medium tall	Haiti
25.	Veti-25	Medium tall	Pune, Maharastra (India)
26.	Veti-26	Medium tall	Kanpur, U.P. (India)
27.	Veti-27	Medium tall	CIMAP, Lucknow, (India)
28.	Veti-28	Medium tall	New Delhi, (India)
29.	Veti-29	Medium tall	Barabanki, U.P. (India)
30.	Veti-30 (DH-1)	Tall	CIMAP, Lucknow, (India)
31.	Veti-31	Medium tall	CIMAP, Lucknow, (India)
32.	Veti-32 (Var. KS-1)	Tall	CIMAP, Lucknow, (India)
33.	Veti-33	Medium tall	Jhansi, U.P. (India)
34.	Veti-34	Medium dwaef	Moradabad, U.P. (India)
35.	Veti-35	Tallest	Kukra/Bankey Ganj, Lakhimpur (Kheri) U.P. (India)
36.	Veti-36 (Var. Gulabi)	Medium tall	CIMAP, Lucknow, (India)
37.	Veti-37	Medium tall	Nimach, M.P. (India)
38.	Veti-38	Medium tall	Trissur (Kerala) (India)
39.	Veti-39	Medium tall	Gandhi Nagar, Gujarat (India)
40.	Veti-40	Tall	Basari Ghat U.P. (India)
41.	Veti-41	Tall	Musanagar, U.P. (India)
42.	Veti-42 (Selection-1)	Tall	CIMAP, Lucknow, (India)
43.	Veti-43	Medium tall	Musanagar, U.P. (India)
44.	Veti-44	Medium tall	Nimach, M.P. (India)
45.	Veti-45	Medium tall	Trissur (Kerala) (India)
46.	Veti-46	Medium tall	Razaganj, Lakhimpur (Kheri), U.P. (India)
47.	Veti-47	Medium tall	Travancore, Kerala, (India)
48.	Veti-48 (Var. Keshari)	Dwarf	CIMAP, Lucknow, (India)
49.	Veti-49	Medium dwarf	CIMAP, Lucknow, (India)
50.	Veti-50	Medium tall	Ghaghraghat, U.P. (India)
51.	Veti-51	Medium tall	Reunion, Island
52.	Veti-52	Medium tall	Chennai, Andhra Pradesh (India)
53.	Veti-53	Medium tall	CIMAP, Lucknow, (India)
54.	Veti-54	Medium tall	CIMAP, Lucknow, (India)

55.	Veti-55	Medium tall	Indonesia
56.	Veti-56	Medium tall	CIMAP, Lucknow, (India)
57.	Veti-57	Medium tall	Bharat Pur, Rajasthan, (India)
58.	Veti-58	Tall	Medium tall
59.	Veti-59 (Var. KS-2)	Tall	CIMAP, Lucknow, (India)
60.	Veti-60 (Var. CIM Vriddhi)	Tall	CIMAP, Lucknow, (India)

Table 1: Origin of 60 accessions of vetiver maintained at CSIR-CIMAP, Lucknow.

Source of variations	d.f.	Mean sum of squares (m.s.s.)			
		Characters			
		Plant height (mts)	Root yield/plot (g)	Essential oil contents (%)	Essential oil yield/plot (g)
Replications	2	0.022	1670.000	0.005	0.111
Treatments	119	0.048**	28136.030**	0.477**	9.470**
Accessions	59	0.058*	27320.750**	0.665**	11.296**
Year	1	0.026**	9616.000**	0.147**	13.844**
Accessions × Year	59	0.040**	29742.030**	0.305**	7.731**
Error	238	0.013	3958.319	0.011	0.379
Total	359				

*-p < 0.05, **-p < 0.01, respectively.

Table 2: Pooled ANOVA for the sixty accessions of two years of Vetiver.

Accession in clusters	I	II	III	IV	V	VI	$\bar{D}^2$	Accessions included in clusters
I (36)	12.20 (3.49)	16.25 (4.03)	35.36 (5.95)	55.02 (7.42)	52.07 (7.22)	207.55 (14.41)	63.08	1, 3, 6, 9, 12, 13, 14, 16, 18, 20, 21, 24, 25, 27, 28, 31, 32, 33, 34, 36, 37, 39, 41, 42, 43, 45, 47, 50, 51, 52, 53, 54, 55, 56, 57, 59
II (14)		7.03 (2.65)	26.63 (5.16)	45.95 (6.78)	73.50 (8.57)	235.77 (15.35)	67.52	2, 11, 15, 17, 19, 22, 26, 35, 38, 44, 46, 48, 49, 58
III (6)			5.48 (2.34)	124.35 (11.15)	144.88 (12.04)	372.10 (19.29)	118.13	4, 5, 7, 8, 29, 30
IV (2)				1.55 (1.24)	38.21 (6.18)	104.82 (10.24)	61.65	40, 60
V (1)					0.00	58.96 (6.68)	61.27	10
VI (1)						0.00	163.20	23

D = $\sqrt{D^2}$ values are in parenthesis; $\bar{D}^2$= average D^2 values

Table 3: Intra (bold) and inter cluster D^2 values of vetiver accessions.

Maximum contribution of genetic divergence characters in per cent towards primary axis (z1 vector) was for essential oil yield g/plot (56.04%) followed by essential oil content (15.83%), root yield/plot (15.70%). The plant height was the least contributor (12.44%) towards genetic divergence. On secondary axis (z2 vector), root yield/plot was the highest contributor (43.00%) followed by plant height (32.46%) and essential oil yield (18.34%), respectively. The oil content was the lease contributor (6.09%) towards genetic diversity. On the other hand on the basis of over all contributions, the essential oil yield/plot (33.58%) was the maximum contributor followed by root yield/plot (29.08%), essential oil content (22.81%). The plant height was the least contributor (14.58%) towards the genetic divergence (Table 4). Coefficient of variation was recorded maximum for essential oil yield (33.58%) and lowest for the trait plant height (14.58%). The critical difference was maximum for root yield and lowest for essential oil content also revealed the presence of high genetic variability in the vetiver accessions (Table 4). The high morphological variation in root lengths in clumps and root web patterns were also noted and presented in figure 4 and figure 5. These traits may be utilized to check the soil erosion and water conservation purpose. Our results are in confirmation of results obtained by Pareek [2], Lal [14], Lal., *et al*. [15], Lal., *et al*. [5] in the vetiver. Therefore, the choice of most economic traits, essential oil yield, root yield/plot, plant height and oil content may be used as a better selection criterion for improvement of vetiver crop. Since the genetic improvement in any crop plant depends solely on the presence of considerable genetic variability, environmental conditions and contributions of various traits towards genetic diversity.

Clusters	Accessions	Plant height (mts)	Root yield/plot (g)	Essential oil content (%)	Essential oil yield/plot (g)
Vetiver accessions in cluster-I	36	1.52	435.62	0.95	3.70
Vetiver accessions in cluster -II	14	1.53	456.68	0.86	3.44
Vetiver accessions in cluster -III	6	1.60	397.25	0.93	3.52
Vetiver accessions in cluster -IV	2	1.55	378.25	1.23	4.69
Vetiver accessions in cluster -V	1	1.72	356.50	0.75	3.25
Vetiver accessions in cluster -VI	1	1.58	371.67	0.79	3.58
Character contributions towards	Primary axis in % (Z_1)	12.44	15.70	15.83	56.04
Character contributions towards	Secondary axis in % (Z_2)	32.46	43.00	6.09	18.34
Character contribution towards genetic diversity	Over all (%)	14.58	29.08	22.81	33.58
Coefficient of variation	C.V. $_{(\%)}$	7.32	14.61	11.46	16.88
Critical difference	C. D, $_{(5\%)}$	0.18	101.20	0.17	0.99
Critical difference	C.D. $_{(1\%)}$	0.24	133.56	0.22	1.31

Table 4: Cluster mean for the four most economic traits of the vetiver accessions.

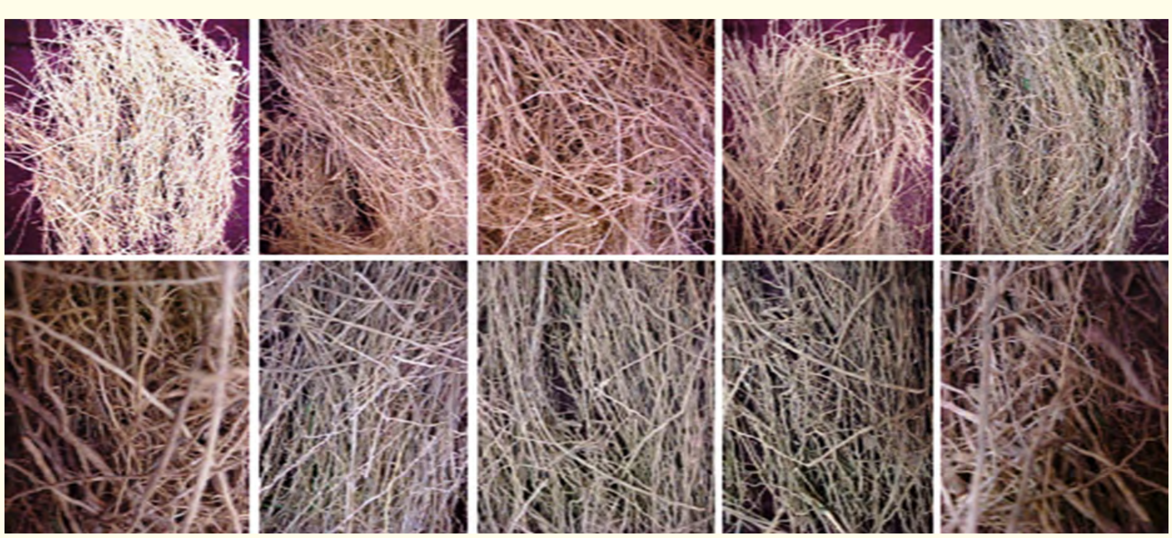

Figure 5: Variability in roots web pattern in the accessions of vetiver.

In its incessant Endeavour to develop high yielding genotypes/varieties of vetiver crop, CSIR-CIMAP, Lucknow has recently developed a new aroma variety of vetiver (CIM-Samriddhi) which is able to produce 35 kg of essential oil/ha as compared to 25 kg/ha produced from the existing variety CIM-Vridhi. The new variety has been developed by using the clonal/clonal selection breeding method. On the basis of their initial performance, superior clones were identified within and between the clones. Superior single plant progenies were multiplied and evaluated under initial evaluation trial (IET) followed by replicated multi-row advanced yield trials for higher oil yields. The essential oil of this new variety will be unique in its aroma due to the high content of its major aroma ingredients > 30% Khusilal (nor-sesquiterpene (C14) aldehydes) and > 19% Khusol (Figure 6-8). This variety will provide at least 20% higher oil yield and profits than the presently popular variety CIM-Vridhi. Moreover, this variety will also provide an opportunity to utilize the unutilized or underutilized lands for productive purposes. The selection for traits essential oil yield, root yield/plot, plant height and oil content may provide good criteria for selection for essential oil yield. In the present study, highly significant differences were observed among the vetiver accessions evaluated indicates existence of good amount of genetic variability with respect of the four most economic characters assessed and offers sufficient probability for the genetic improvement in vetiver accessions and results in the development of superior variety like CIM Samriddhi. Traits like essential oil yield, root yield and essential oil content were the major contributors to genetic divergence. Further, the selection Veti-30 (DH-1) followed by Veti-12 (CIM Samriddhi), Veti-42 (Selection-1), and Veti -60 (CIM Vridhi) can be exploited for commercial exploitation in vetiver.

Figure 6: Newly released variety CIM Samriddhi plants, roots and differential molecular profiling for of vetiver (Chrysopogon zizanioides (L.) by using ISSR primer.

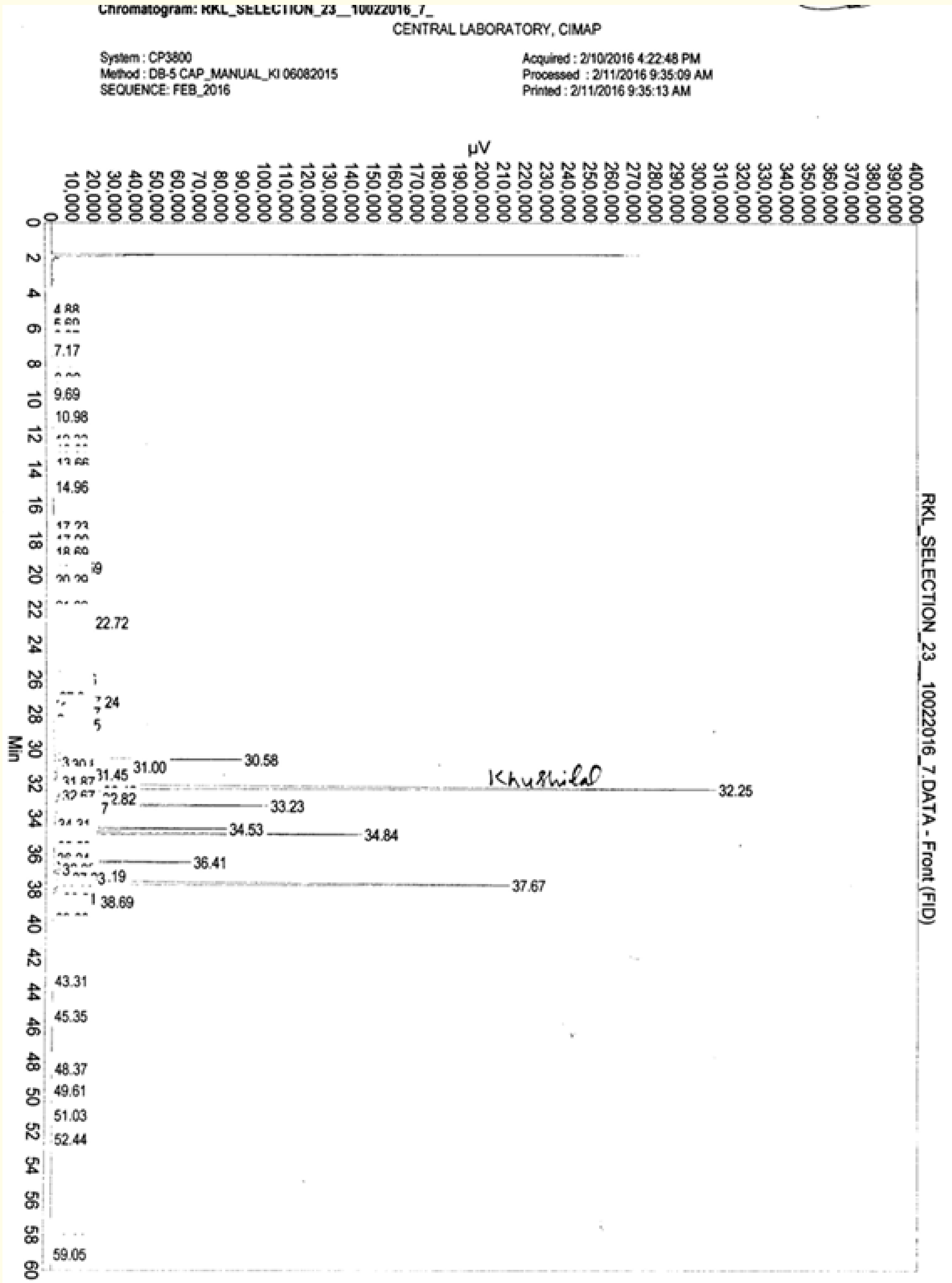

Figure 7: Chromatogram of essential oil of variety CIM Samriddhi.

Figure 8: Variety CIM Samriddhi of vetiver released by Sri Narendra Damodar Modi Honorable Prime Minister of India for commercial cultivation.

Conclusion

Multivariate analysis was performed for knowing the genetic diversity of 60 vetiver accessions using four most economic traits. The accessions/genotypes were grouped into six different clusters in order to understand genetic variation character contribution of various yield components in vetiver. The genotypes Veti-30 (DH-1) followed by Veti-12 (CIM Samriddhi), Veti-42 (Selection-1), and Veti -60 (CIM Vridhi) showed high genetic divergence and selection of these accessions may result in high genetic gain for essential oil yield characters. Traits like essential oil yield, root yield and essential oil content were the major contributors to genetic divergence. Further, these selection namely, Veti-30 (DH-1) followed by Veti-12 (CIM Samriddhi), Veti-42 (Selection-1), and Veti -60 (CIM Vridhi) can be exploited for commercial exploitation in vetiver.

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Bibliography

1. Hussain A., *et al*. "*Vetiveria zizanioides* (L.) Nash Vetiver or Khus. Status Report on Genetic Resources on Important Medicinal and Aromatic Plants in South Asia". CIMAP, Lucknow, India (1984): 273-304 (behalf of International Board of Plant Genetic Resources Rome).
2. Pareek SK. "Germplasm collection and evaluation of vetiver". In: Rana RS, Singh B, Kopar MN, Mathur R, Kochar S, Dahoon SS. (Eds.), Plant Genetic Resources Exploration, Evaluation and Maintenance. NBPGR, New Delhi, (1994): 275-281.
3. Virmani OP and Datta SC. "*Vetiveria zizanioides* (L.) Nash". *Indian Perfumer* 19 (1975): 95-73.
4. Lavania UC. "Primary and secondary centres of origin of vetiver and its dispersion". In: Second International Proceedings on Vetiver and the Environment Conference, Vetiver, Phetchaburi Province Thailand (2000): 432-434.
5. Lal RK., *et al*. "Phylogenetic Relationships, Path and Principal Component Analysis for Genetic Variability and High Oil Yielding Clone Selection in Vetiver (*Vetiveria zizanioides* L.) Nash". *Journal of Plant Genetics and Breeding* 2.1 (2018): 105-113.

6. Lal RK., *et al*. "Genetic diversity in germplasm of vetiver grass, *Vetiveria zizanioides* (L.) Nash". *Journal of Herbs, Spices and Medicinal Plants* 5.1 (1997a): 77-84.

7. Lal RK., *et al*. "Varietal selection for high root and oil analysis in vetiver *Vetiveria zizanioides* (L.) Nash". *Journal for Medicinal and Aromatic Plant Science* 19 (1997b): 1-4.

8. Lal RK., *et al*. "Genetic variability and exploitation in vetiver grass, *Vetiveria zizanioides* (L.) Nash". *Journal for Medicinal and Aromatic Plant Science* 21 (1999): 963-968.

9. Clevenger JF. "Apparatus for the determinations of volatile oils". *Journal of the American Pharmacists Association* 17.4 (1928): 345-349.

10. Panse VG and Sukhatme PV. "Statistical Methods for Agricultural Workers". 2nd edition. Indian Council of Agricultural Research, New Delhi (1967).

11. Mahalanobis PC. "On the generalized distance in statistics". *Proceedings of the National Academy of Sciences* 2 (1936): 49-55.

12. Rao CR. "Advanced Statistical Methods in Biometrical Research". 1st edition. John Wiley and Sons. Inc., New York (1952): 1-160.

13. Singh RK and Chaudhary BD. "Biometrical Methods in Quantitative Genetic Analysis". Kalyani Publisher, New Delhi (2014): 1-252.

14. Lal RK. "On genetic diversity in germplasm of vetiver (*Vetiveria zizanioides* L. Nash)". *Industrial Crop and Products* 43 (2012): 93-98.

15. Lal RK., *et al*. "Genetic variability and character associations in vetiver (*Vetiveria zizanioides* L. Nash)". *Industrial Crop and Products* 49 (2013): 273-277.

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