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Vetiver DNA-Fingerprinted Cultivars: Effects of Environment on Growth, Oil Yields and Composition

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Abstract

Twenty one accessions of vetiver (*Vetiveria zizanioides* (L.) Nash, sterile, oil type) and Khus (*V. zizanioides*, fertile, non-oil type) were analyzed by the use of random amplified polymorphic DNAs (RAPDs). Nineteen of the accessions clustered strongly around the cultigen, Sunshine. Three accessions, Khus, Northern India, Kassel, Germany, and Guang Dong, China clustered loosely and were not closely related to the sterile oil producing vetivers. Thirteen of the vetiver accessions were grown in test plots in Florida, USA, Nepal and Portugal. The largest growth was recorded in Nepal, followed closely by Florida and by the cooler, Mediterranean site in Portugal. No single genotype (DNA cultivar) grew best in every plot. Oil yields (% oil/dry wt.) were highest in Nepal and Portugal. Oil yields ranged from 0.29% to 9.61%. Essential oil production (g/plant) was highest in Nepal and Florida and ranged from 0.02 to 4.17 (g/plant). Analyses of variation among the major compounds is discussed.

Key Word Index

Geographic variation, vetiver, *Vetiveria zizanioides*, *Poaceae*, *Chrysopogon zizanioides*, random amplified polymorphic DNAs, RAPDs, essential oils.

Introduction

Vetiver (*Vetiveria zizanioides* (L.) Nash, syn. *Chrysopogon zizanioides* (L.) Roberty) has been utilized in many parts of the world to control soil erosion. Hedges of the non-seeding vetiver provide an effective living dam against erosion (1) and this technique is now in use in more than 100 countries. The origin of the non-seeding vetiver is not known. However, *V. zizanioides* seems to have originated in the area from India to Vietnam and its fragrant roots have been used for centuries for mats and perfumes (1).

Adams and Dafforn (2) examined 121 accessions of vetiver and found that 86% appeared to be from a single clone (no variation in the DNA examined). That clone was named Sunshine (after its collection site in Sunshine, LA). Included in that analysis were plants from Haiti and Reunion that appear to be Sunshine. So the commercial vetiver cultigen used for commercial essential oil production is Sunshine or a very similar cultigen. This work was expanded by Adams et al. (3) to include closely related genera (*Chrysopogon* and *Sorghum*). Based on this and morphological data, Veldkamp (4) combined *Vetiveria* and *Chrysopogon* under *Chrysopogon*. Although this led to the recognition of *Chrysopogon zizanioides* (L.) Roberty as the correct name for *Vetiveria zizanioides* (L.) Nash, in this paper we will continue to use both names for clarity. Analysis of additional cultigens from Bangkok (5) revealed that Sunshine and its allied cultigens form the bulk of the cultigens in the world.

Based on these results, 13 different DNA types were cloned and planted in test plots with distinctly different environments (Florida, USA, Kathmandu, Nepal and Lagos, Algarve, Portugal). These test plots have now been harvested and the essential oils removed. This paper reports on their growth, analysis of these oil yields and DNA fingerprinting of additional accessions of vetiver.

Vetiver oil from Haiti has been exhaustively studied recently (6-10), but the composition is so complex (most GC

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Table I. Vetiver cultivars identified by DNA fingerprinting

Code	Accession #	Lab #	Cultivar, Source
SS*	VET-MRL-001	7749	cv. 'Sunshine', USA. Louisiana
HF*	VET-MB-01	8029	cv. 'Huffman', USA, Florida
CP*	VET-LW-0001	8048	cv. 'Capitol', Louisiana
SL*	VET-RN-001	7951	cv. 'Sri Lanka', Colombo
SM*	VET-IMZ-AGA	8349	cv. 'Malawi', Lilongwe
CR*	VET-JM-PV1	8076	cv. 'Costa Rica', Puerto Viejo
PnB*	VET-RGG-PA-B	7720	cv. 'Panama B', Panama
ZO*	VET-SJC-2	7775	cv. 'Zomba' Malawi, Zomba
MA*	VET-TGML-001	8244	cv. 'Malaysia', Spain, (Malaysia)
AV*	VET-TGAVC-002	8245	cv. 'AVC', ex. Spain, AVC Corp. USA
KR*	VET-TGKN-003	8246	cv. 'Karnataka', Spain, (Malaysia)
ST*	VET-TGBB-005	8248	cv. 'Sabak Buntar', Spain, (Malaysia)
PB*	VET-TGPB-006	8249	cv."Parit Buntar', Spain, (Malaysia)
B1	VET-NS-001	8339	cv."Songkla 1', Thailand
B2	VET-NS-002	8340	cv. 'Surat Thani', Thailand
B3	VET-NS-003	8341	ex. Sri Lanka, via Thailand
SH	VET-TGSB-004	8247	cv. 'Sabah', Spain, (Malaysia)
RC	VET-JM-RON	8348	cv. 'Ronca', Costa Rica
GU	VET-RM-001	8852	cv. 'Talo', Guam
PH	VET-ABE-001	8889	ex. Philippines
KA	VET-TDH-001	8822	cv."Euro', ex. Kew, ex. Kassel, Germany
GD	VET-XH-001	8161	ex. Guang Dong, Xia Hanping, China
KH	VET-SCRC-001	8035	ex. N. India, Khus, seedy vetiver

those marked with an asterisk (*) were planted in test plots for evaluation

peaks contained two to four components), that general, routine analyses of vetiver oils are probably not possible.

Recently, Dethier et al. (11) reported that soil fertilization increased oil yields dramatically without changing the composition. This suggests that there may be improved methods to grow vetiver for oil production. So analysis of oils of different genotypes seems very timely.

Experimental

Specimens were collected as given in Table I. The leaf samples were shipped desiccated in silica gel (12). The DNA from vetiver is not preserved well in either fresh or air dried leaves. Interim preservation of the leaves in silica gel is necessary but preservation in ethanol is preferred for transit. Upon receipt, all the materials were transferred to 95% ethanol for 48 h (22°C), the ethanol was removed and the leaves were kept frozen until analyzed. DNA was extracted using the hot CTAB protocol (13) with the addition of 1% (w/v) PVP and Proteinase (150 µg).

The Polymerase Chain Reactions (PCR) were performed in a volume of 15 µL containing 50 mM KCl, 10 mM Tris-HCl (pH 9), 2.0 mM $MgCl_2$, 0.01 % gelatin and 0.1% Triton X-100, 0.2 mM of each dNTPs, 0.36 µM primers, 0.3 ng genomic DNA, and 0.6 unit of Taq DNA polymerase (Promega). A control PCR tube containing all components, but no genomic DNA, was run with each primer to check for contamination.

The following 21, 10-mer primers (numbers from U. British Columbia project) were used: 116, TAC GAT GAC G; 131, GAA ACA GCG T; 134, AAC ACA CGA G; 153, GAG TCA CGA G; 204, TTC GGG CCG T; 212, GCT GCG TGA C; 218, CTC AGC CCA G; 239, CTG AAG CGG A; 244, CAG CGA ACC G; 249, GCA TCT ACC G; 250, CGA CAG TCC C; 265, CAG CTG TTC A; 268, AGG CCG CTT A; 327, ATA CGG CGT C; 346, TAG GCG AAC G; 347, TTG CTT GGC G; 352, CAC AAC GGG T; 375, CCG GAC ACG A; 391, GCG AAC CTC G; 431, CTG CGG GTC A; 432, AGC GTC GAC T. These primers gave several bright bands, did not have any false bands (in the controls) and were proven to be reproducible in replicated analyses. DNA amplification was performed in an MJ Programmable Thermal Cycler (MJ Research, Inc.). The thermal cycle was: 94°C (1.5 min) for initial strand separation, then 40 cycles of 38°C (2 min), 72°C (2 min), 91°C (1 min). Two additional steps were used: 38°C (2 min) and 72°C (5 min) for final extension. Amplification products were analyzed by electrophoresis on 1.5% agarose gels and detected by staining with ethidium bromide. The gels were photographed under UV light with Polaroid film 667. pGEM DNA (Promega) was used as a molecular weight marker. The RAPD bands were scored by molecular weight and assigned a code based on primer number prefix and molecular weight category. In addition, the RAPD band intensity was scored as: 0 = no band; 4 = faint ; 5 = medium; 6 = bright band, in reference to a gray tone standard (14).

These data were coded into a matrix of taxa by character values. Similarity measures were computed using absolute character state differences (Manhattan metric), divided by the maximum observed value for that character over all taxa (= Gower metric; (15,16). Division by the character state range was tried and found to be less informative than using the maximum observed character value (i.e., including zero in the

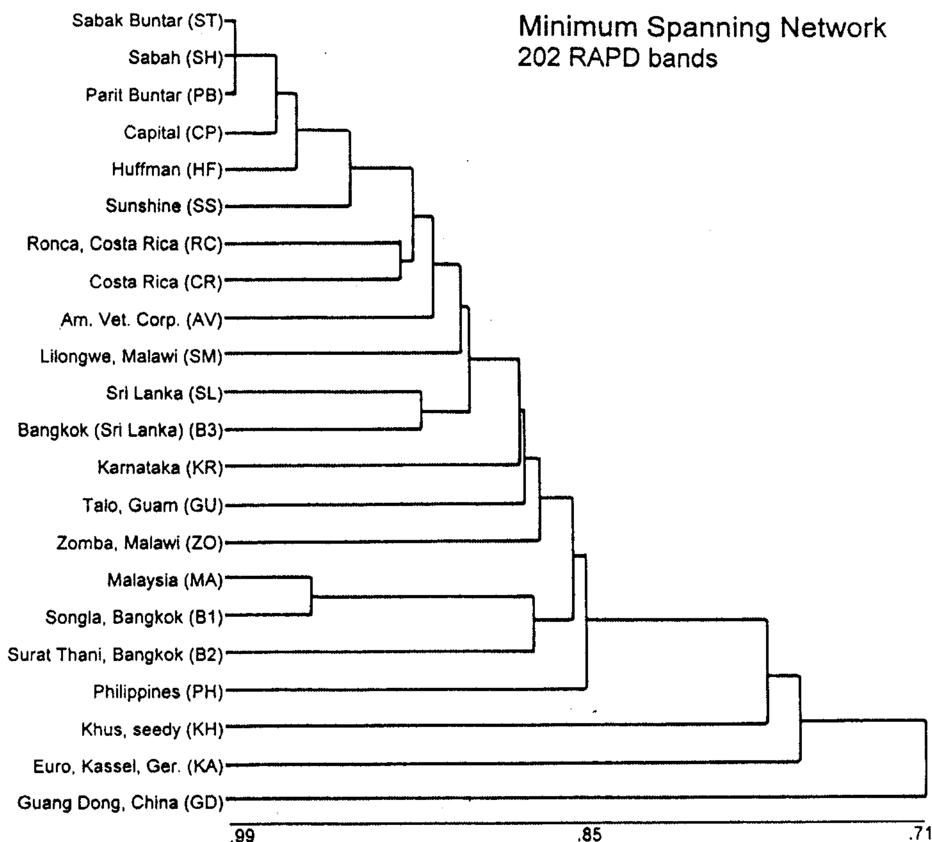

Figure 1. Minimum spanning network based on 202 RAPD bands; most of the accessions cluster with Sunshine—see text for discussion

range). Principal coordinate analysis (PCO) of the similarity matrix follows Gower (17) by program PCO3D.

The vetiver roots were dug and air-dried in the shade for four to five days, then sent to the lab where they were kept at -20°C until distilled. The roots were steam distilled for 24 h using a circulatory Clevenger-type apparatus (18) The oil samples were concentrated (ether trap removed) with nitrogen and the samples stored at -20°C until analyzed. The extracted roots were oven dried (48 h, 100°C) for determination of oil yields.

The oils were analyzed on a Hewlett-Packard 5973 MSD, directly coupled to a HP5980 gas chromatograph. EI mass spectra were collected at 70 eV ionization voltage over the mass range m/z 41-425. Oil samples of 0.1 µL (5% concentration) were injected and split 1/10. Analytical conditions: Column: J & W DB-5, (0.26 mm x 30 m, 0.25 µm film thickness); carrier gas: helium at 1 mL/min; injector temperature 220°C; split ratio: 10:1; oven programming: initial temperature: 60°C, gradient 3°C/min, final temperature: 246°C. The percentage of each compound are TIC values. Identifications were made by library searches of our volatile oil library (19) coupled with retention time data of reference compounds.

A two-way ANOVAs were run for plant growth, % oil yields, oil yields/plant, and % khusinol. In cases with missing data these were removed from the two-way ANOVA. One-way ANOVAs were calculated for between locations and between genotypes, then Fisher's pairwise comparisons were used to determine significant differences.

Results and Discussion

Previously, we reported that the vetiver from Panama [Panama B (2,3,5)] was very distinct. We re-extracted new materials of 'Panama B' and found it to be essentially the same as 'Sunshine', the common vetiver planted around the world (2). So we did not include it in the current assessment of similarities among the DNA types of vetiver. Figure 1 shows that most of the DNA cultivars cluster loosely with 'Sunshine' in the midst of the cluster. Bangkok (ex Sri Lanka) clusters loosely with 'Sri Lanka' cultivar (Figure 1). The Malaysia (MA) cultivar clusters closely with Songla, Bangkok (B1) and then with the second Bangkok sample (B2, Figure 1). The most distinct accessions (Figure 1) are Euro (KA) and Guang Dong (DG) and the seedy, Khus (KH).

The overall picture of the relationships is depicted by Principal Coordinates Analysis (PCO, Figure 2). Notice that most of the cultivars cluster strongly. Guang Dong (GD) and Khus (KH) are most distinct. We do not know if the oil of 'Euro' is intermediate between Khus (seedy) and the sterile

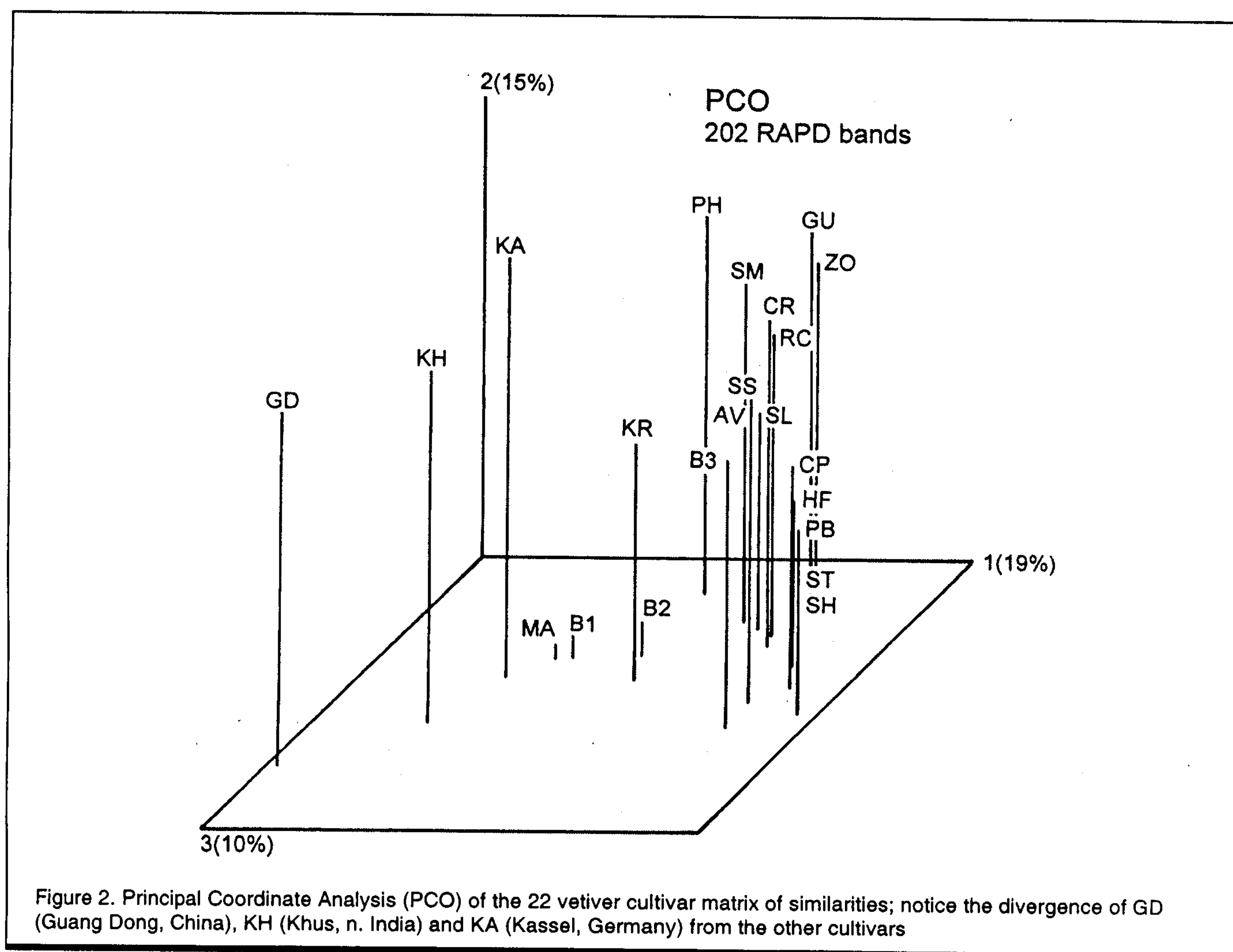

Figure 2. Principal Coordinate Analysis (PCO) of the 22 vetiver cultivar matrix of similarities; notice the divergence of GD (Guang Dong, China), KH (Khus, n. India) and KA (Kassel, Germany) from the other cultivars

'Sunshine' cultivars (as shown in Figure 2), because 'Euro' is only recently being planted in test plots for evaluation.

Test plots for 13 cultivars (Table I) were established in May, 1999 at Kathmandu, Nepal (sub-tropical, 1,300 m elev.), Ft. Meyers, FL (ECHO, tropical, 10 m) and Lagos, Algarve, Portugal (Mediterranean, ca. 20 m elev.). The plots were complete randomized design with three reps per accession. Vetiver was planted in rows, 1 m between rows, with 0.5 m spacing between plants within the rows. The plants were watered during the first month for establishment and not watered thereafter. No fertilizer was applied. The plots were kept weed free by hand weeding. Measurements on plant height and basal area were taken quarterly. Because vetiver oil appears to reach a maximum after about 18 months (11), one individual of each DNA cultivar was dug up and the roots harvested in October 2000.

Vetivers grew best in Nepal followed by Florida and then Portugal, where there is a decidedly cool winter (Table II). The death of a cultivar in test plots (Table II), was not connected with inhospitable conditions, but due to stress in sending the plantlets to these sites. Interestingly, no single cultivar grew best in all three plots. Costa Rica (CR), Panama B (PnB) and Parit Buntar (PB) were significantly larger in the challenging Portugal test plot (Table II). Whereas, Huffman (HF) and Capital (CP) grew poorest in Portugal. Sri Lanka (SR) proved to be difficult to grow in the greenhouse where the original plantlets were produced in Texas. So the planting materials for SR were generally not as robust as those of other cultivars. Recently, it was observed (in the Florida plot) that stems of KRN (Karnataka) had fallen over and produced plantlets at the nodes. This is not a desirable characteristic for erosion control plantings in farmlands, so KRN should not be used for soil erosion control. Overall, the vetiver genotypes grew only about half as large in Portugal as in Nepal or Florida (Table II). There is significant interaction between genotypes and locations ($P < .001$) implying that growth of some genotypes are well suited to certain locations.

The percent oils yields varied among cultivars and plots (Table III). In general, the highest yields were from Nepal and Portugal and much lower in Florida (Table III). The Florida plot had a very high water table (ca. 30 cm below the surface) and this may have affected the oil yields and/ or root growth. All of the high yielding cultivars (above 5.7%) clustered strongly in the 'Sunshine' cluster (Figures 1 and 2). In contrast, the low yielding cultivars (KR, ZO, SM, MA, in Nepal, Table III) are more removed from the basic 'Sunshine' cluster

Table II. Comparison of growth (in cm) among Nepal, Portugal and Florida test plots

	Nepal	Portugal	Florida	#Signif.
Huffman (HF)	289.3^{3e}	96.5^{1b}	223.0^{2de}	**
Sabak Bernam, Mal.(ST)	286.7^{2e}	died	205.0^{1cd}	**
Sunshine (SS)	275.7^{3d}	116.0^{1b}	204.7^{2c}	**
Capital (CP)	278.0^{3d}	90.0^{1a}	235.0^{2g}	**
Am. Vet. Corp. (AV)	277.3^{3d}	112.3^{1b}	223.0^{2ef}	**
Panama B (PnB)	266.7^{3c}	142.0^{1e}	220.3^{2ef}	**
Costa Rica (CR)	265.0^{3c}	140.7^{1e}	198.3^{2c}	**
Karnataka, Mal. (KR)	261.3^{2c}	died	223.3^{2ef}	**
Malawi, Africa (SM)	248.7^{3b}	120.7^{1cd}	218.7^{2ef}	**
Zombia, Malawi(ZO)	247.7^{3b}	120.0^{1bc}	228.3^{2fg}	**
Malaysia (MA)	239.7^{2a}	117.0^{1b}	122.7^{1a}	**
Sri Lanka (SL)	died	died	216.3^{de}	
#Significance	**	**	**	
Average height	266.2^{1}	118.6^{3}	207.9^{2}	**

#significance: ** = 0.01, * = 0.05; values not sharing the same number superscript in a row are significantly different at the significance indicated; values not sharing the same letter superscript in a column are significantly different at the significance indicated; genotype x location interaction: F= 44.25, P <.001

Table III. Comparison of oil yields (% oil, oven dry wt. basis) among Nepal, Portugal and Florida plots

	Nepal	Portugal	Florida	#Signif.
Costa Rica (CR)	8.31^{2f}	8.02^{2c}	4.41^{1e}	**
Am. Vet. Corp. (AV)	8.05^{2ef}	9.61^{3d}	4.53^{1ef}	**
Huffman (HF)	7.97^{2cf}	7.75^{2c}	5.14^{1fg}	**
Sabak Bernam, Mal.(ST)	7.68^{2ef}	died	3.42^{1d}	**
Sunshine (SS)	7.36^{2ef}	7.10^{2c}	4.78^{1efg}	**
Parit Buntar (PB)	7.21^{3de}	3.96^{1a}	5.42^{2g}	**
Capital (CP)	6.41^{2cd}	5.38^{12b}	5.02^{1fg}	NS
Panama B (PnB)	5.73^{1c}	7.57^{2c}	4.86^{1efg}	**
Malawi, Africa (SM)	3.55^{2g}	3.84^{2a}	2.42^{1c}	*
Karnataka, Mal. (KR)	1.59^{1a}	died	1.64^{1b}	NS
Zombia, Malawi(ZO)	1.54^{1a}	4.08^{3a}	2.42^{2c}	**
Malaysia (MA)	0.86^{2a}	NT	0.29^{1a}	*
Sri Lanka (SL)	died	died	4.65^{ef}	
#Significance	**	**	**	
Averages/ location	5.52^{12}	6.36^{2}	3.77^{1}	*

#significance: ** = 0.01, * = 0.05, NS = not significant; values not sharing the same number superscript in a row are significantly different at the significance indicated; values not sharing the same letter superscript in a column are significantly different at the significance indicated; genotype x location interaction: F = 14.12, P <.001

(Figure 1). There is no doubt that the vetiver carried around the world and planted for essential production (often failing for various economic reasons) is 'Sunshine' and its allies. And for a good reason, they are high yielding cultivars with the acceptable vetiver oil constituents. There is a significant interaction between genotypes and locations for oil yields (P <.001), indicating that oil yields may be influenced by the environment at different locations.

When one examines the actual yield per plant, the situation changes. In Nepal, the Sabak Bernam (SB) cultivar was clearly superior (4.17 g/plant), followed by Sunshine, Huffman and Costa Rica (Table IV). The low yielding (% yield) cultivars (SM, ZO, KR, MA) were also low producing (g/plant, Table IV). In Portugal, Panama B (PnB) and American Vetiver Corp. (AV) were highest yielding (Table IV), but lower than in Nepal or Florida. In general, the yields/plant were greatest in Nepal, then Florida and much lower in Portugal (Table IV). In Portugal, this reflects the much lower biomass production (Table II). The best cultivars for oil production in all three sites are Sunshine and Huffman. Although, no one cultivar performed best in all three environments. There is a significant interaction between genotypes and locations for oil yield per plant (P <.001), suggesting that some genotypes are well suited for some locations.

The oil of vetiver is so complex (10) that a simple GC/MS study of the oil is not possible at this time. Khusinol is the largest component of vetiver oil. A two-way ANOVA of genotypes and locations (Table V) reveals significant variation between locations and within genotypes at each location. The highest concentrations of khusinol were in Nepal, followed by Florida, with Portugal being less (Table V). In general, the percent composition of khusinol is less variable than the oil

Table IV. Comparison of total oil yield/plant (g/plant) among Nepal, Portugal and Florida plots

	Nepal	Portugal	Florida	*Signif.
Sabak Bernam, Mal.(SB)	4.17^{2e}	died	1.10^{1bc}	**
Sunshine (SS)	2.90^{3d}	0.81^{1bc}	1.85^{2ef}	**
Huffman (HF)	2.81^{3d}	0.77^{1b}	2.07^{2f}	**
Costa Rica (CR)	2.59^{3d}	0.82^{1b}	1.64^{2de}	**
Parit Buntar (PB)	2.41^{3cd}	0.87^{1b}	1.68^{2e}	**
Am. Vet. Corp. (AV)	2.11^{2c}	1.23^{1cd}	1.28^{1cd}	**
Capital (CP)	2.10^{2c}	0.56^{1ab}	1.70^{2e}	**
Panama B (PnB)	1.38^{1b}	1.28^{1d}	1.53^{1de}	NS
Malawi, Africa (SM)	0.33^{1a}	0.89^{2bc}	0.84^{2b}	*
Zombia, Malawi(ZO)	0.34^{1a}	0.38^{1a}	0.74^{2b}	*
Karnataka, Mal. (KR)	0.20^{1a}	died	0.30^{1a}	NS
Malaysia (MA)	0.09^{2a}	NT	0.02^{1a}	**
Sri Lanka (SL)	died	died	1.26^{cd}	
*Significance	**	**	**	
Averages/ location	1.79^{2}	0.85^{1}	1.23^{2}	*

*significance: ** = 0.01, * = 0.05, NS = not significant; values not sharing the same number superscript in a row are significantly different at the significance indicated; values not sharing the same letter superscript in a column are significantly different at the significance indicated; genotype x location interaction: $F = 11.65$, $P < .001$

Table V. Comparison of khusinol content among DNA genotypes and test plots in Nepal, Portugal and Florida

	Nepal	Portugal	Florida	*Signif.
Malawi, Africa (SM)	31.42^{3j}	25.33^{2h}	22.72^{1e}	**
Costa Rica (CR)	28.13^{3i}	17.33^{1d}	21.14^{2c}	**
Parit Buntar (PB)	27.64^{3h}	14.52^{1a}	27.06^{2j}	**
Zombia, Malawi(ZO)	25.22^{2g}	26.03^{3i}	24.95^{1i}	**
Huffman (HF)	22.72^{2f}	18.63^{1f}	22.74^{3e}	**
Am. Vet. Corp. (AV)	21.89^{2e}	18.27^{1e}	24.01^{3h}	**
Sabak Bernam, Mal.(SB)	21.83^{1e}	-	23.12^{2f}	**
Sunshine (SS)	21.25^{3d}	17.04^{1c}	20.25^{2b}	**
Malaysia (MA)	21.03^{1c}	-	24.72^{2j}	**
Capital (CP)	20.32^{1b}	21.50^{2g}	22.30^{3d}	**
Karnataka, Mal. (KR)	19.12^{2a}	-	17.93^{1a}	**
Panama B (PnB)	18.92^{2a}	16.35^{1b}	23.62^{3g}	**
Sri Lanka (SL)	-	-	27.11^{k}	
*Significance	**	**	**	
Averages/ location	23.29^{2}	19.44^{1}	23.21^{2}	*

*significance: ** = 0.01, * = 0.05; values not sharing the same number superscript in a row are significantly different at the significance indicated; values not sharing the same letter superscript in a column are significantly different at the significance indicated; genotype x location interaction: $F = 3.03$, P .001

yields (Table II). Note that, in Nepal for example, the oil yields varied from 0.86 to 8.31% (Table III), but the % khusinol varied only from 18.92 to 31.42% (Table V). This is the general case of essential oils that percent concentration varies less than absolute yields (g/g).

It is interesting to examine variation among major components. Table VI lists the cultivars by plots with nine of the main components. Vetivenic acid is not normally a large component, but its occurrence was so unusual, that it is included. All of the cultivars had khusimol (14-31%), (E)-isovalencenol (10-16%), β-vetivenone (2 - 6%) and α-vetivone (3-6%) (Table VI) as main components. However, vetivenic acid was generally absent in Portugal and often absent or very small in Nepal. In contrast, vetivenic acid was present in all but Huffman (HF) in Florida and often in considerable amounts (8.4% in Sunshine, Table VI). The unknown major sesquiterpenes shown in Table VI are KI1626, M^+222(4%), 43(100), 55(30), 67(32), 81(95), 93(34), 105(32), 161(50), 189(15), 204(20), 207(22); KI1801, M^+220(12%), 41(70), 55(45), 67(30), 79(70), 91(100), 105(90), 121(90), 131(25), 145(35), 161(20), 173(8), 187(17), 202(22); KI1834, M^+218(50%), 41(67), 53(35), 65(28), 77(59), 91(100), 105(67), 119(48), 133(30), 148(33), 161(50), 176(27), 189(20), 203(31). KI1626 is generally two to three times as large in the Portugal plot as in Nepal or Florida for all cultivars (Table VI). The cool, dry climate favors this sesquiterpene.

KI1801 is not much effected by growing site (Table VI). KI 1834 is larger in the Florida plot for several cultivars and unaffected in other cultivars (Table VI).

Several samples of commercial vetiver oils were also analyzed and tremendous variation was found in these samples (Table VII). Khusimol and (E)-isovalencenol, major compo-

Table VI. Comparison of composition of major compounds among Nepal, Portugal and Florida plots for each DNA cultivar and with commercial vetiver oils

Clone/location	Component								
	KI1626	vetsol	khsmol	isovol	KI1801	β-vet	veta	KI1834	α-vet
Sunshine									
Nepal	4.5	3.0	21.2	16.1	3.4	3.7	3.1	3.1	3.9
Portugal	7.9	3.5	17.0	16.0	4.0	3.5	-	3.5	3.7
Florida	2.3	3.6	20.2	16.5	3.3	4.0	8.4	2.1	5.1
Huffman									
Nepal	4.0	2.9	22.7	14.3	3.0	3.3	0.5	2.8	3.2
Portugal	7.4	3.3	18.6	15.3	3.8	3.5	t	3.4	4.5
Florida	2.7	3.5	22.7	15.3	3.1	3.4	-	3.0	5.0
Capitol									
Nepal	2.0	3.5	20.3	16.5	3.2	3.6	0.6	3.5	4.6
Portugal	5.3	3.5	21.5	15.7	4.0	3.2	0.8	3.8	4.6
Florida	3.0	3.3	22.3	14.6	3.1	3.0	1.1	2.6	4.7
Sri Lanka									
Florida	3.1	3.0	27.1	11.7	3.3	4.4	2.2	2.1	3.2
Malawi, Africa									
Nepal	1.1	3.2	31.4	14.0	3.2	3.0	-	4.1	4.4
Portugal	6.1	3.4	25.3	12.1	2.9	2.2	-	4.0	3.7
Florida	4.1	2.8	22.7	10.0	2.4	5.8	4.3	3.1	3.2
Costa Rica									
Nepal	4.5	2.7	28.1	12.8	2.5	3.4	-	2.8	4.2
Portugal	5.6	3.0	17.3	11.6	3.4	3.1	-	2.8	3.4
Florida	2.6	3.0	21.1	12.7	2.5	7.1	6.2	1.7	4.6
Panama B									
Nepal	2.2	3.2	18.9	14.7	3.0	3.6	t	3.6	5.7
Portugal	6.2	3.7	16.35	15.6	3.7	3.2	-	3.3	4.3
Florida	2.4	3.1	23.6	13.4	2.7	5.7	4.9	1.6	4.7
Zombia, Malawi									
Nepal	2.0	3.2	25.2	11.7	2.6	3.6	-	3.6	5.7
Portugal	5.7	3.3	26.0	13.2	3.4	2.5	0.5	4.8	4.2
Florida	4.0	3.4	24.9	10.5	2.4	3.8	1.0	6.7	3.2
Malaysia									
Nepal	1.8	3.6	21.0	11.9	2.1	2.0	-	4.5	5.1
Florida	4.7	2.6	24.7	9.8	1.8	1.3	0.4	2.6	2.3
American Vetiver Corp. (AVC)									
Nepal	2.3	3.0	21.89	14.6	2.8	3.4	t	3.4	5.4
Portugal	7.4	3.5	18.27	15.7	4.1	3.3	-	3.3	4.1
Florida	2.3	3.6	24.0	13.5	2.7	4.6	0.1	5.6	4.1
Karnatka, Malaysia									
Nepal	1.4	9.2	19.1	11.3	1.9	3.6	-	1.6	4.1
Florida	2.8	11.0	17.9	14.8	2.4	3.3	t	1.7	5.2
Sabak Bernam, Malaysia									
Nepal	2.3	2.7	21.8	12.8	2.5	6.7	1.8	2.6	4.0
Florida	1.7	2.9	23.1	12.2	2.5	5.3	7.8	0.1	5.0
Parit Buntar, Malaysia									
Nepal	1.9	2.9	27.6	14.1	3.0	3.7	2.0	3.2	4.9
Portugal	6.3	3.4	14.5	11.5	2.7	2.4	-	2.6	3.0
Florida	2.0	3.4	27.0	11.8	2.3	4.3	1.6	5.7	4.2

vetsol = vetiselinenol; khsmol = khusimol; isovol = (E) isovalencenol; β-vet = β-vetivone; veta = vetivenic acid; α-vet = α-vetivone

nents on all of the genotypes, were large in some commercial oils and small in others. Khusimol ranged from 36.0% (China, cultivated in Guang Dong) to 4.5% (France, Alternative Therapies Lab.). (E)-Isovalencenol ranged from 16.1% (Haiti, Texaroma) to 2.1% (Java, Djasula). Most of the commercial oils contained 1.2-2.9% vetivenic acid except the Guang Dong sample, (25.2%, Table VII). Nootkatone, often reported in vetiver oil (4.3-10.2% in 11) was found to be only a trace or absent, except for one Java, Belanusa sample that contained 0.8%. Dethier et al. (11) reported from 4.0 to 9.2% isokhusimol in their samples from Burundi, but we found no isokhusimol, but rather considerable amounts of (E)-isovalencenol (Table VII), which they do not report. Weyerstahl et al. (10) also reported large amounts of (E)-isovalencenol (eremophila-1(10),7(11)-dien-2α-ol) in Haitian vetiver oil. It is interesting to note the differences

Table VII. Comparison of commercial vetiver oils with Sunshine oil, from Florida

Source	KI1626	vetsol	khsmol	isovol	KI1801	β-vet	veta	KI1834	α-vet
Sunshine, FL	2.3	3.6	20.2	16.5	3.3	4.0	8.4	2.1	5.1
Unknown, A. C.[1]	3.0	3.5	14.4	8.2	1.3	3.2	1.0	1.1	4.2
China, cult., GD[2]	-	1.8	36.0	15.4	1.1	-	25.2	1.4	4.2
El Salvador	1.0	3.3	16.4	9.2	1.7	5.3	2.9	1.7	3.8
France, A. Th.[3]	t	3.8	4.5	4.7	-	3.7	7.6	0.8	6.6
Haiti, A. Th.[3]	2.3	4.1	12.5	11.8	2.2	3.5	-	0.9	4.8
Haiti, Berje	1.7	4.2	17.1	11.7	2.2	3.0	1.2	1.3	4.4
Haiti, Texarome	1.4	5.2	22.9	16.1	2.5	4.8	-	0.8	6.6
Indonesia, Berje	-	2.2	7.9	2.4	1.8	4.9	1.8	0.6	3.5
Japan, 'Ohito'[4]	-	1.8	24.8	4.7	t	3.4	4.4	3.4	3.3
Java, Djasula	3.5	2.6	10.6	4.2	0.8	5.3	1.6	1.0	3.8
Java, Djasula	3.1	2.0	7.3	2.1	1.8	4.8	1.5	0.8	4.0
Java, Belanusa	-	2.2	8.5	3.3	1.7	5.5	2.4	0.8	4.7
Java, Belanusa	2.3	2.5	12.9	6.5	0.7	5.4	1.4	1.4	6.1

vetsol = vetiselinenol; khsmol = khusimol; isovol = (E) isovalencenol; β-vet = β-vetivone; veta = vetivenic acid; α-vet = α-vetivone.
[1]Aura Casia, Weaverville, CA
[2] Guang Dong, China, cultivated, thanks to Xia Hanping
[3]Alternative Therapies Laboratory, Canada
°Originally from Japan, cultivated in Taiwan, thanks to Yue-wen Wang

Table VIII. Comparison of winter survival at Gruver, TX, elev. 1000 m, Lat. 36° 15' 54"N, Longitude 101° 24' 21"W (NT = not tested)

	1999-2000	2000-2001
Minimum air temperature	-13.3°C	-15.5°C
Min. soil temp, 2" depth	-4.6°C	-5.0°C
Min. soil temp. 6" depth	0°C	-0.5°C
Sunshine (SS)	ok	died
Huffman (HF)	ok	1 shoot, mostly dead
Capital (CP)	ok	1 shoot, mostly dead
Sri Lanka (SL)	NT	NT
Malawi, Africa. (SM)	ok	5 vigorous shoots
Costa Rica (CR)	ok	died
Panama B (PnB)	ok	4 vigorous shoots
Zombia, Malawi(ZO)	ok	1 shoot, mostly dead
Malaysia (MA)	died back	died
Am. Vet. Corp. (AV)	ok	6 vigorous shoots
Karnataka, Mal. (KR)	died to 1 shoot	died
Sabak Bernam, Mal.(SB)	ok	1 shoot, mostly dead
Parit Buntar (PB)	ok	1 shoot, mostly dead

between two lots of oil from Djasula (Java) and another two lots from Belanusa (Java) (Table VII).

There has been a search for cold-hardy vetiver for use in soil erosion control in Europe and North America. A test plot was planted in Gruver, Texas, USA (elev. 1000 m, Lat. 36° 15' 54"N, Long. 101° 24' 21"W for the 13 genotypes. Most survived air temperatures of -13.3°C and soil temperatures of -4.6°C (Table VIII). However, the following year we experienced an air temperature just slightly colder (-15.5°C), soil (-5°C) which killed most of the cultivars (Table VIII). Only Malawi (SM), Panama B (PnB) and American Vetiver Corp. (AV) showed good survival and these were severely affected. It appears that about -15°C is the temperature limit for these cultivars.

Following our survey of DNA fingerprints of vetivers of the world (2,3,5), we have assembled the known types and when cultivated in common gardens, their oils are relative uniform compared to the commercial oils analyzed. It would appear that additional factors must be responsible for the variations seen in these commercial oils. Extraction differences, additional genetic variation, unknown edaphic factors and/or oil adulterations are likely involved in causing these differences.

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