DNA ANALYSIS OF THE GENETIC DIVERSITY OF VETIVER AND FUTURE SELECTIONS FOR USE IN EROSION CONTROL

Robert P. Adams
Plant Biotechnology Center
Baylor University, Gruver, Texas, USA

Abstract

Random Amplified Polymorphic DNAs (RAPDs) were used to examine accessions of vetiver (*Vetiveria zizanioides* (L.) Nash) and related taxa from its region of origin and around the world. It appears that only one *V. zizanioides* genotype, "Sunshine", accounts for almost all germplasm used outside South Asia. Additional RAPD analyses revealed that several other non-fertile accessions are distinct genotypes. This germplasm diversity holds promise for reducing the vulnerable genetic uniformity in what is now essentially a pantropical monoculture of an economically and environmentally important plant resource. Evaluation trials of these accessions are in progress. When *Vetiveria* cultivars and species were compared with *Chrysopogon* and *Sorghum* species, the *Vetiveria* taxa clustered by themselves but were most similar to *Chrysopogon*. Analysis of vetiver cultivars and putative *V. nemoralis* from Thailand suggested that *V. nemoralis* is a distinct taxon. It was found that vetiver (and other grasses) have DNases (proteins) that are not irreversibly denatured by desiccation. DNA from air-dried leaves was often found to be degraded beyond use. A modified interim field preservation method is suggested. Material submitted for DNA analysis should be small (actively growing) leaves, harvested fresh and immediately placed into ethanol overnight and then shipped in either ethanol or activated silica gel.

Introduction

The introduction of new plants into the environment by man is often in the form of monocultures. These monoculture crops are extremely inbred for factors such as yield, uniform flowering and height, and cosmetic features of the products. This narrow genetic base has resulted in several disastrous crop failures. For example, Ireland's potato (*Solanum tuberosum* L.) famine of 1846 resulted in the emigration of a quarter of its population. This was due to the fact that Irish potatoes had no resistance to *Phytophthora infestans*, the late blight fungus (Plucknett et al. 1987). The lack of resistance can be traced to the lack of genetic diversity in Irish potatoes, which had been multiplied using clonal materials from just two separate South American introductions to Spain in 1570 and thence to England in 1590 (Hawkes 1979). When the late blight fungus attacked the Irish potatoes, there were no individuals with resistance genes among these two potato lines.

A more recent example is the southern maize leaf blight (fungus, *Helminthosporium maydis*) in 1970 in the United States. Because almost all maize (*Zea mays* L.) in the United States was of hybrid origin and contained the Texas cytoplasmic male sterile line, maize fields presented an unlimited, extremely narrow gene base habitat for the fungus. By late summer 1970, plant breeders were scouring maize germplasm collections in Argentina, Hungary, Yugoslavia and the United States for resistant sources (Plucknett et al. 1987). Nurseries and seed fields were used in Hawaii, Florida, the Caribbean and Central and South America to incorporate the resistance into hybrid maize in time for planting in the spring of 1971 (Ullstrup 1972). Without these genetic resources this technological feat would not have been possible. Both the potato and maize examples show the susceptibility of a very narrow genetic base to an ecological disaster.

During the past 10 years, a tall, pantropical grass has been used in many parts of the world to control soil erosion: vetiver (*Vetiveria zizanioides* (L.) Nash). Hedges of the non-seeding vetiver provide an effective living dam against erosion (National Research Council 1993; World Bank 1994) and this technique is now in use in more than 160 countries. The exact origin of the non-seeding vetiver is not known. However, *V. zizanioides* seems to have originated in the area from India to Viet Nam and its

fragrant roots have been used for centuries for mats and perfumes (National Research Council 1993).

Kresovich et al. (1994) reported on clonal variation of vetiver using RAPDs. They found RAPD patterns were very stable within clones and they were able to distinguish between “Huffman” and “Boucard” plants, and the USDA PI 196257 introductions. In addition, they found that each of the three USDA PI 196257 accessions (#1, 2, 3) were genetically different. They concluded that RAPDs would be very useful for identifying truly new and/or different sources of diversity.

Elite germlines of *Vetiveria zizanioides* (L.) Nash have long been cultivated for their fragrant roots, which contain the essential oil of vetiver. This oil is clearly distinguished chemically and in commerce from *Khus* oil, which comes from natural (fertile) populations of *V. zizanioides* in the Ganges Plain of North India (CSIR 1976). The oil of vetiver (commercial, essential oil type) has long been produced pantropically via vetiver cuttings. Within the past decade, vetiver occurrence has increased enormously through widespread plantings (over 100 countries) to form hedges for stabilizing soil and controlling waterflow.

One of the most desirable features of hedgerow vetiver is that it is non-fertile and must be grown from cuttings (clumps of rootstock). Thus, because it does not reproduce by seeds, it is a very well behaved grass throughout the tropics and subtropics. It has not escaped cultivation and become a weed. However, the mere fact that it is always distributed by cuttings could lead to the widespread cultivation of a single clone. This would be extremely dangerous. If an insect or disease became adapted to a clone, the adaptation could spread and decimate millions of erosion control terraces of vetiver. In order to investigate this matter, we assembled leaf materials from cultivated vetiver from around the world and analysed these accessions using RAPDs (DNA fingerprints). In addition, other *Vetiveria* species and two putatively related genera, *Chrysopogon* Trin. and *Sorghum* Moench, were analysed in an effort to begin to understand the potential germplasm pool for future selections.

The preservation of plant specimens by silica gel desiccation for subsequent DNA analysis use is now routine (Adams et al. 1992). The Missouri Botanical Garden Herbarium even has a DNA bank that consists of silica gel-dried materials (Miller and Schmidt 1998). It is thought that DNases are denatured by the extreme desiccation produced by dry silica gel (Adams et al. 1992). The most common DNA extraction protocol used in plant science appears to be the hot CTAB method (Doyle and Doyle 1987). In this and most other protocols, EDTA is a component because of its ability to chelate Mg^{2+} ions, which are often needed by DNases (Ogawa and Kuroiwa 1985). However, the assumption that all DNases can be inhibited by EDTA is not warranted. For example, in *Clamydomonas reinhardtii*, six DNases were found and each of these required Ca^{2+} for activation (Ogawa and Kuroiwa 1985). The DNases were little affected by the amount of Mg^{2+} ions. In tobacco, the DNases did not appear to need any specific ions for activity and were inhibited by Mg^{2+} (Zilberstein et al. 1987). Two DNases were found in wheat seedlings (Jones and Boffey 1984): one required Mg^{2+} and the other was activated by EDTA. Jones and Boffey (1984) concluded: “Thus, EDTA alone will not protect DNA from cleavage during its isolation from wheat seedlings.”

The DNases are even more complex in rice. Sodmergen et al. (1991) found that rice contained 13 DNases with the following ion requirements: five Ca^{2+}, four Zn^{2+} and four Mg^{2+} dependent. If EDTA only chelates Mg^{2+} ions, it would not be effective in inhibiting these DNases.

We recently received some vetiver (*Vetiveria*, Poaceae) samples from Madagascar that were shipped in silica gel in resealable plastic bags. The blue indicating crystals had turned partially pink (implying some rehydration had occurred). The DNAs from these samples were very degraded. It appears that the DNases in vetiver may be reactivated by the addition of water. In fact, we have encountered difficulty in obtaining good DNA from vetiver by grinding directly in CTAB (Adams et al. 1998). It has been necessary to grind in liquid nitrogen and then regrind in CTAB. We thought this problem was due to the fibrous nature of the *Vetiveria* leaves (particularly when dry) which caused it to be difficult to grind to a fine powder in CTAB. However, we experienced difficulty in obtaining DNA of uniform quality, even when extracting fresh vetiver leaves which are easy to grind. It seemed plausible that the DNases were reactivated by water in the extraction buffer and that EDTA was ineffective against these DNases.

Because different plant species apparently produce different kinds of DNases, it seems that a more general method for DNase inactivation is needed. Previously, we reported on the effectiveness of various alcohols in preserving plant specimens (Flournoy et al. 1996). The alcohols apparently precipitated the proteins (including DNases) and, thus, protected the DNA. Ethanol was found to be the most effective alcohol tested (Flournoy et al. 1996). I am including in this paper some recent information (Adams et al. 1999) on the effects of ethanol on DNases from both fresh and silica gel-dried materials from several plant families that resulted in a more general method for the interim preservation of plants.

Material and Methods

Specimens were collected as given in Tables 1-4. Leaves were shipped fresh, air dried, or desiccated in silica gel (Adams et al. 1992). The DNA from vetiver was not preserved well in either fresh or air-dried leaves. Upon receipt, all the materials were frozen until analysed. DNA was extracted using the hot CTAB protocol (Doyle and Doyle 1987) with the addition of 1% (w/v) PVP and Pronase E (150ìg). Grinding in hot CTAB (60^{o}C) in a hot mortar and pestle was somewhat effective for some accessions, but most accessions yielded larger molecular weight DNA and greater yields when the tissue was ground in liquid nitrogen and then placed in hot CTAB (unless preserved in ethanol). Often yellowed leaves yielded degraded DNA when ground directly in hot CTAB, but yielded more high molecular weight DNA (20-50 kbp) when ground in liquid nitrogen and then incubated in hot CTAB.

PCR was performed in a volume of 15 µl containing 50 mM KCl, 10mM Tris-HCl (pH 9), 2.0 mM $MgCl_2$, 0.01 % gelatin and 0.1 % Trit 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 ten-mer primers (numbers from the University of British Columbia project) were used: 134, AAC ACA CGA G; 184, CAA ACG GAC C; 212, GCT GCG TGA C; 218, CTC AGC CCA G; 234, TCC ACG GAC G; 239, CTG AAG CGG A, 244, CAG CCA 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. These primers gave several bright bands, did not have false bands (in the controls) and were proved to be reproducible in replicated analyses. DNA amplification was performed in an MJ Programmable Thermal Cycler (MJ Research, Inc.). The thermal cycle was: 94^{o}C (1.5 min) for initial strand separation, then 40 cycles of 38^{o}C (2 min), 72^{o}C (2 min), 91^{o}C (1 min). Two additional steps were used: 38^{o}C (2 min) and 72^{o}C (5 min) for final extension. Amplification products were analysed 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 grey te standard (Adams and Demeke 1993). It might be noted that analyses using simple 0 = absent and 1 = present scoring gave very similar results, except that the eigenroots were not as strongly loaded on the first few axes, implying that the information content was less than when the 0-6 scale was used. In replicated analysis, we found that the relative band intensity was very reproducible in our lab. In our RAPD analyses, every primer generated at least one very bright band (level 6). Over the past several years we have screened over 250 primers and selected about 15 primers that we use routinely. Any primer that did not generate at least one level-6 band (very bright) was not used in the analyses. In addition, if the PCR amplification does not result in at least one level-6 band, the sample is reanalysed in triplicate. Invariably, upon reamplification all three reanalyses result in at least one level-6 band. The brightest of the triplicate samples is then re-electrophoresed with the other samples. This iterative approach results in a set of very similar amplifications for each sample. Thus, the relative intensities are preserved.

Several factors may be responsible for the presence of faint bands: single copy DNA for faint bands vs. multiple DNA copies for bright bands; tertiary folding of DNA with cross bonding making the DNA less amenable to PCR amplification; and competitive interactions between bands for TAQ enzymes and substrates during amplification.

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; Gower 1971; Adams 1975). 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 range). Principal coordinate analysis (PCO) of the similarity matrix follows Gower (1966) by a DOS-based program, PCO3D (available for PC computers from RPA).

For the DNases study, leaves from fresh spinach (*Spinacia oleracea* L.), broccoli (*Brassica oleracea* L.), alfalfa sprouts (*Medicago sativa* L.) were purchased locally. Juniper (*Juniperus virginiana* L.) leaves were collected from trees cultivated near the laboratory. Sorghum (*Sorghum bicolor* (L.) Moench.), wheat (*Triticum aestivum* L.), maize (*Zea mays* L.) and rice (*Oryza sativa* L.) seeds were secured locally and germinated to obtain seedlings.

Plant material was ground in a micro-mortar in 100 ìl of either ddwater, CTAB or ethanol. The sample was then incubated for 20 min at 37^{o}C as a DNase activity assay (in the case of ethanol, 350 ìl of ddwater was added before incubation). Then 350 ìl of hot CTAB (Doyle and Doyle 1987) was added to the CTAB or ethanol extracted samples. Proteinase (150 ìg, Sigma P6911) was added and all the samples were incubated an additional 30 min at 60^{o}C. The DNA was precipitated by the addition of 2 volumes of ethanol (rather than the use of 2/3 volume of isopropanol, as in Doyle and Doyle 1987). DNA was separated on 0.6-% agarose gels by electrophoresis (20 min, 100 v, 10 v/cm) with ethidium bromide in the gel and buffer. DNA quantities and qualities were estimated by comparison with serial dilutions of genomic mouse DNA (Sigma D-0144) and lambda Hind III markers. Gels were photographed over short-wave UV light using a Polaroid direct-screen camera (DS34). In addition, all the extracted DNAs were subjected to PCR-RAPDs (Adams et al. 1998) using a standard RAPD primer (UBC 268) to check for the quality of the DNAs.

Results and Discussion

Initial Screening

An initial screening (Adams et al. 1998) of accessions (n=53) using 222 banding patterns found almost no variation among cultivated materials. The pattern obtained by primer 268 is typical of that obtained using primers 184, 239, 249, 327 and 346. Essentially no variation was detected in an initial 27 accessions for outside South Asia, except for a quite similar accession from Malawi.

A second series of accessions (n=68) was analysed (Adams et al. 1998) running only one highly discriminating primer (268). This analysis, while revealing additional variation in non-fertile types, reinforced patterns that form several distinct genetic clusters. These groupings are validated by correspondence to botanical taxa and field observations (reports of fertility) (Table 1).

Of 60 total samples submitted from 29 countries outside South Asia, 53 (88 %) were a single clone of *Vetiveria zizanioides* (Adams et al. 1998). At least two thirds of these samples were first accessioned from traditional, in-country sources, i.e. oil producers, herbalists, botanical gardens and other planted sites, and therefore may be considered representative of ex situ vetiver populations. Because vetiver is vegetatively propagated, it appears that one single essential-oil clone (which we are denoting as "Sunshine" because of accession priority) is densely distributed throughout the tropics. Its introduction was done certainly before WWII and most likely before the 20^{th} century. For instance, vetiver has been in the United States since at least the early 19^{th} century, although the earliest authenticated germline identifications are currently 'Vallonia', South Africa, via Mauritius, c. 1900, M. Robert; 'Monto', Australia, 1930s, P. Truong; 'Sunshine', USA, 1960s, E. LeBlanc; and MY044693 and MY081268, Venezuela, 1982, O. Rodriguez; (information from The Vetiver Network members). Such a consistent identity in a spatially and temporally scattered distribution implies that virtually all of the *Vetiveria zizanioides* outside South Asia could be the single 'Sunshine' genotype, which today certainly dominates soil stabilization and waterflow-control usage.

These concerns led us to look for additional non-fertile germplasm to broaden the genetic base for erosion control projects (Adams et al. 1998). A second solicitation for accessions of vetiver, related *Vetiveria* species and, presumably related, *Chrysopogon* and *Sorghum* species was made (Table 2).

Thirteen primers were run on the 18 accessions in Table 2. The RAPD analysis yielded 222 bands that were coded. A minimum spanning network revealed that the vetivers and related species cluster together.

However, notice that the *Chrysopogon fluvus* and *C. gryllus* are quite distinct, but are linked first by their similarity to a vetiver (Nepal, NP; and *V. elongata*, EN, respectively). The *Sorghum* species cluster together and form a separate group.

Principal coordinate analysis (PCO) was run on these 18 Operational Taxonomic Units (OTUs) (Table 2). The first five eigenroots extracted 21.55, 11.22, 9.71, 7.47, and 7.00 % of the variance among the eighteen OTUs. The eigenroots appear to asymptote after the first five roots. The first principal coordinate separates *Sorghum* from *Vetiveria* and *Chrysopogon* supporting its taxonomic distinctiveness. The second axis separates the *V. elongata* (EB, EN), *V. filipes* (FP) and the Panama vetiver (PB) from the other vetiver accessions. The third axis principally separates the *Chrysopogon* gryllus from all the other accessions. However, notice that *C. gryllus* is most similar to *V. zizanioides* from Nepal (0.69, NP), whereas *Chrysopogon fulvus* is most similar to *V. elongata* (0.72). The similarity between *C. fulvus* and *C. gryllus* is only 0.67. The fact that these two *Chrysopogon* species are each more similar to *Vetiveria* taxa than to each other indicates that some taxonomic revision is warranted between *Chrysopogon* and *Vetiveria.*

The *Sorghum* taxa were added as an out-group to *Vetiveria*, and that is exactly as they appear: similar to each other, but rather distant from *Vetiveria.* The *Vetiveria* taxa cluster fairly tightly (similarities between 0.81 and 0.90). To examine sub-clustering among the *Vetiveria*, one can graph additional principal coordinates. However, because *Chrysopogon* and *Sorghum* principally accounted for coordinates 1 and 3 and part of coordinate 2, it is reasonable to remove these taxa and recompute PCO using only the *Vetiveria* taxa.

After the *Chrysopogon* and *Sorghum* taxa were removed from the data set, PCO was performed using the remaining fourteen *Vetiveria* OTUs. The first five eigenroots removed: 19.01, 12.82, 10.11, 9.34 and 8.78 % of the variance among the *Vetiveria* OTUs, before appearing to asymptote. Most striking in the ordination is the distinctness of the vetiver from Panama, site B (PB). It is as dissimilar to the other *V. zizanioides* (SS) as are the recognized species, *V. elongata* (EN, EB) and *V. filipes* (FP). As there are no recognized *Vetiveria* species native to the new world, the Panama accession may be an introduced plant from the old world, or perhaps *Chrysopogon pauciflorus* (Chapm.) Vasey, which is reported from Cuba and Florida.

The vetiver OTUs from Bangladesh, India and Nepal form a notably tight cluster. The putative *V. nigritana* from Malawi (NG) is loosely (0.81) associated with *V. zizanioides* (from northern India). Whereas, *V. zizanioides* cv. 'Graft'(GR) from Australia is most similar to "Sunshine" (SS), albeit at the same level of similarity as the putative *V. nigritana* is to vetiver from India (0.81).

PCO using only the nine putative *V. zizanioides* OTUs yielded eight eigenroots with no apparent asymptote. This indicates that there is little clustering among these OTUs. Ordination shows that three of the OTUs from India (In1, In2, In8) do form a tight cluster, but the other OTUs are fairly disjoint. There is some clustering of the Bangladesh (BG), India (In1, In2, In8, InP, In10) and Nepal (NP) OTUs. 'Sunshine' (SS) is divergent from the main North India group, and "Graft" is even more divergent. It is interesting to note that, apparently, only 'Sunshine' is non-seeding, although Graft has low seed fertility (1-3%, Paul Truong, pers. comm.). Several additional accessions had similar patterns to other OTUs (Table 2).

Genetic Diversity within *Vetiver zizanioides*

An early report on variation in vetiver from Thailand (Strifah et al. 1998) was recently updated (Adams et al. 2000) using 217 RAPD bands. A minimum spanning tree revealed (Adams et al. 2000) that three main groups are present in the data set: 'Sunshine' vetiver, the six putative *V. nemoralis* from Thailand (B4-B9) and the single Panama B accession from Panama. Note that AV (American Vetiver Corp.) and KR (cv. Karnataka from Malaysia) show the greatest differences in the 'Sunshine' vetiver complex. All of these accessions are non-fertile.

PCO analysis of this similarity matrix removed 69.4% of the variance among the accessions by nine eigenroots. These eigenroots accounted for 20.4, 10.0, 8.2, 6.7, 5.6, 5.2, 4.8, 4.3 and 4.2 % of the variance. The eigenroots appear to asymptote after the 5^{th} root. A 3-d ordination reveals that coordinate

1 separated the putative *V. memorials* (Thailand) from all the other accessions (Fig. 7). The second axis seems to separate the *V. zizanioides* from Thailand (B1-B3) from other accessions. The Panama B (PB) accession was separated by the third axis. Clearly, the accessions of *V. nemoralis* from Thailand form a group and this indicates that this group may warrant some taxonomic recognition.

In order to more clearly discern the inter-relationships among the *V. zizanioides* accessions, the putative *V. nemoralis* accessions and the Panama B accession were removed from the data set and a new PCO was performed. This PCO resulted in removing 82.1% of the variance among accessions by the first nine eigenroots: 20.0, 12.0, 10.2, 9.3, 7.7, 7.0, 6.0, 5.1 and 4.8%. The first ordination shows that the 'Sunshine' accession (SS) occupies a central position with the three Thailand accessions (B1-B3) ordinated away from the main portion of the vetiver accessions. Several Malaysian accessions tended to cluster as a group. The second coordinate shows the unique nature of one of the Malaysian accessions (ML) and the Karnataka (KR) accession. It should be noted that the relationships shown in the minimum spanning network were unaffected by removing the *V. nemoralis* and Panama B accessions, because they were not most similar to any of the *V. zizanioides* accessions.

Accessions AV (American Vetiver Corp.) and KR (Karnataka, Malaysia) were heavily loaded onto coordinates 4 and 5, respectively. Ordination using axes 1, 4 and 5 reveals that these OTUs are quite distinct. Note particularly that AV is not clustering close to 'Sunshine' (SS). This distant relationship to SS is, of course confirmed in the minimum spanning network. It should be noted that there is often distortion when only three axes are used in ordination. In this case, it takes a separate ordination using axes 4 and 5 to portray the variation.

Several points were revealed from that study (Adams et al. 2000). New sources of germplasm should be accessioned from the Thailand materials (B1-B3). The Thailand *V. nemoralis* accessions should be further investigated as to their taxonomic status (species or infraspecific taxon?). In contrast, to the previous work (Adams et al. 1998), this more robust DNA analysis (217 bands) shows the AV (American Vetiver Corp.) accession to be a source of germplasm that is quite distinct from 'Sunshine'. The Panama B plants need to be more thoroughly taxonomically investigated. Some of the accessions are so closely related that only one type should be included in test plot evaluation (e.g., SB, SH, PT, HF), if time and money are constraints.

In order to diversify the current germplasm, we are establishing test plots in several countries using the following accessions: Sunshine (SS), Songkhla (B1), Surat Thani (B2), Sri Lanka (B3) via Thailand, Malaysia (ML), Karnataka (KR), American Vetiver Corp. (AV), Hoffman (HF), Capital (CP), Colombo, Sri Lanka (SL), Costa Rica (CR) and Zomba, Malawi (ZM).

Preservation of Vetiver for DNA Analysis

We (Adams et al. 1999) have found that all of the species examined contained DNases that degraded the DNA when the ground material was incubated in ddwater for 20 min at 37^{o}C (Table 4), except juniper, in which case, the DNA was only partially degraded but completely degraded after 24 hours at 37^{o}C. Note particularly that preservation in silica gel does not irreversibly inactivate DNases. In every case, except broccoli, the DNA in silica gel-dried leaves was degraded when the leaves were ground in water and incubated (Table 4). Thus, it appears that when shipping materials, one must be very careful that they are not rehydrated either during transit or subsequent to extraction.

All of the non-grasses yielded very good DNA when ground in CTAB and then incubated in CTAB (20 min, 37^{o}C). In contrast, CTAB was either not very effective or ineffective in protecting the DNA for most of the grasses (Table 4). Only the fresh maize and fresh wheat yielded very good DNA (20-50 kbp) when ground in CTAB and incubated. Fresh sorghum and silica-dried wheat yielded good (some degraded DNA) under these conditions.

However, even the most recalcitrant species (vetiver and rice) yielded very good DNA (Table 4) when the materials were first ground in ethanol and then incubated in ddwater (20 min, 37^{o}C). It seems that the ethanol, in precipitating the proteins, has also irreversibly denatured the DNases.

All of the samples that yielded good or very good DNAs (Table 4) gave good bands by PCR-RAPD, whereas those with poor or degraded DNAs either failed to amplify or produced variable bands.

The grinding of plant material in a small quantity of ethanol, before grinding in the extraction buffer (CTAB in this instance), would seem to be a general method for the inactivation of DNases, regardless of their requirements for Mg^{2+}, other ions or no ions.

In summary, it appears that one should immerse the fresh vetiver leaves in ethanol overnight to completely denature the DNases, then either ship in ethanol or pack the leaves in activated silica gel in very tightly closed containers before shipping for DNA analysis.

Acknowledgements

Thanks to many colleagues who have worked on this project. They include: Mark Dafforn, Yang Fei, Ziao-qiang Ma, Naresh Pandey, Nitsri Sangduen, Lakhan Singh, Pattana Srifah, Yerlon Turuspekov, Jeff Veldkamp and Ming Zhong. This research was supported by funds from Baylor University, the Conservation, Food and Health Foundation and the Wallace Genetic Foundation. I also want to thank the numerous collaborators in The Vetiver Network who contributed samples of vetiver tissue.

References

Adams, R.P. 1975. Statistical character weighting and similarity stability. Brittia 27: 305-16.

Adams, R.P. and Demeke T. 1993. Systematic relationships in *Juniperus* based on random amplified polymorphic DNAs (RAPDs). Taxon 42: 553-71.

Adams, R.P.; Do, N.; and Chu, G.L. 1992. Preservation of DNA in plant specimens from endangered tropical species by desiccation. *In:* Conservation of Plant Genes: DNA Banking and *in vitro* Biotechnology, pp. 135-152. Academic Press, New York, USA.

Adams, R.P.; Zhong, M.; Srifah, P.; and Sangduen, N. 2000. DNA genetic diversity of *Vetiveria zizanioides* (Poaceae). Phytologia (in press).

Adams, R.P.; Zhong, M.; Turuspekov, Y.; Dafforn, M. R.; and Veldkamp, J. F. 1998. DNA fingerprints reveal clonal nature of Vetiver (*Vetiveria zizanioides* L. (Nash) and sources of potential new germplasm. Molec. Ecol. 7: 813-8.

Adams, R.P.; Zhong, M.; and Fei, Y. 1999. Preservation of DNA in plant specimens: Inactivation and reactivation of DNases in field specimens. Molec. Ecol. 8: 681-683.

CSIR. 1976. Vetiveria. pp. 451-457. *In:* The Wealth of India, Vol. X. Publications and Information Director, CSIR, New Delhi.

Doyle, J.J. and Doyle, J. L. 1987. A rapid DNA isolation procedure for small quantities of fresh tissue. Phytochem. Bull. 19: 11-5.

Flournoy, L.E.; Adams, R.P.; and Pandey R.N. 1996. Interim and archival preservation of plants specimens in alcohols for DNA studies. BioTechniques 20: 657-60.

Gower, J.C. 1966. Some distance properties of latent root and vector methods used in multivariate analysis. Biometrika 53: 315-328.

Gower J. C. 1971. A general coefficient of similarity and some of its properties. Biometrics 27: 857-874.

Hawkes, J.G. 1979. Genetic poverty of the potato in Europe. *In:* Proc. Conf. Broadening the Genetic Base of Crops, A.C. Zeven and A.M. van Harten, eds., Wageningen, Netherlands.

Kresovich, S.; Lamboy, W.F.; Li, R.; Ren, J.; Szewc-McFadden, A.K.; and Bliek, S.M. 1994. Application of molecular methods and statistical analyses for discrimination of accessions and clones of vetiver grass. Crop Sci. 34: 805-809.

Jones, M.C. and Boffey, S.A. 1984. Deoxyribonuclease activities of wheat seedlings. Federation of European Biochem. Soc. 174: 215-218.

Miller, J.; and Schmidt, H. 1998. The Missouri Botanical Garden's DNA Bank. *In:* Conservation of Plant Genes III: Conservation and Utilization of African Plants. (eds. Adams R. P. and Adams J. E.). pp. 175-82. Missouri Botanical Garden Press.

National Research Council. 1993. Vetiver Grass: A Thin Green Line Against Erosion. National Academy Press, Washington, DC.

Ogawa, K.; and Kuroiwa, T. 1985. Nuclease C: Polymorphism of Calcium-dependent nucleases in Clamydomonas reinhardtii. Plant Cell Physiol. 26: 481-491.

Plucknett, D.L.; Smith, N.J.H.; William, J.T.; and Anishetty, N.M. 1987. Gene Banks and The World's Food. Princet University Press, Princet, New Jersey, USA.

Sodmergen, K.; Kawano, S.; Tano, S.; and Kuroiwa, T. 1991. Degradation of chloroplast DNA in second leaves of rice *(Oryza sativa)* before leaf yellowing. Protoplasma 160: 89-98.

Srifah, P.; Ruanjaichon, V.; and Sangduen, N. 1998. The use of random amplified polymorphic DNA for classification of *Vetiveria* spp. in Thailand. Proc. ICV-1, pp.141-146. ORDPB, Bangkok.

Ullstrup, A.J. 1972. The impacts of the southern maize leaf blight epidemics of 1970-1971, Ann. Rev. Phytopath. 10:37.

World Bank. 1994. Vetiver Grass Network. The World Bank, Asia Technical Dept., Washingt, DC.

Zilberstein, A.; Koch, T.; Altschuler, Y.; Lers, A.; and Zamir, A. 1987. Characterization of DNase activity in tobacco leaf extracts. Plant Science 52: 57-65.

Table 1. Preliminary classification of vetiver accessions by DNA fingerprinting.
A = pattern based on 6 primers: 184, 239, 249, 268, 327 and 346
B = pattern based only on primer 268
Fertile codes: N = no, Y = yes, F = fully, L = low, + = confirmed, - = assumed, ? = unknown.
* = botanically verified at the species level

	Accession #	Lab #	Species	Source (other locations)	Fertile?
Vetiveria zizanioides (L.) Nash Sunshine clone (S) (= Haiti, Monto, Boucard, Huffman, Vallonia)					
S^{A}	VET-RPA-7655	7655	V. zizanioides	Haiti, Massif de la Selle, 1600 amsl	N+
S^{A}	VET-RPA-7659	7659	V. zizanioides	Haiti, Marigot, 8 amsl	N+
S^{A}	VET-RPA-7660	7660	V. zizanioides	Haiti, Jacmel, 3 amsl	N+
S^{A}	VET-RPA-7661	7661	V. zizanioides	Haiti, Jacmel, 3 amsl	N+
S^{A}	VET-RPA-7663	7663	V. zizanioides	Haiti, Massif de la Selle, 820 amsl	N+
S^{A*}	VET-PT-1A	7711	V. zizanioides cv. 'Monto'	Australia, Queensland	N+
S^{A*}	VET-PT-1B	7712	V. zizanioides cv. 'Fiji'	Australia, Queensland (Fiji)	N+
S^{A*}	VET-PT-1D	7714	V. zizanioides	Australia, Queensland (Western Australia)	N+
S^{A*}	VET-PT-1E	7715	V. zizanioides	New Guinea	N+
S^{A}	VET-RGG-PA-A	7719	V. zizanioides	Panama, site A	N+
S^{A}	VET-RGG-CR-A	7721	V. zizanioides	Costa Rica, San Jose	N+
S^{A*}	VET-MR-VAL1	7722	V. zizanioides cv. 'Vallonia'	South Africa, Natal	N+
S^{A}	VET-OSR-1.0	7729	V. zizanioides	Venezuela, Maracay (flowers some)	N+
S^{A}	VET-DEKN-1001	7730	V. zizanioides	Aneityum Island, Pacific	N+
S^{A}	VET-DEKN-1003	7731	V. zizanioides	Efate Island, Pacific	N+
S^{A}	VET-DEKN-1002	7732	V. zizanioides	Atiu Island, Pacific	N+
S^{A}	VET-DEKN-1004	7733	V. zizanioides	Mangaia Island, Pacific	N+
S^{A}	VET-GVB-001	7742	V. zizanioides cv. 'Boucard'	USA, Texas, (Haiti or Guatemala)	N+
S^{A}	VET-MJ-F1	7747	V. zizanioides	USA, North Carolina	N+
S^{A}	VET-MJ-F2	7748	V. zizanioides	USA, North Carolina	N+
S^{A*}	VET-MRL-0001	7749	V. zizanioides cv. 'Sunshine'	USA, Louisiana	N+
S^{A}	VET-MRD-0001	7750	V. zizanioides cv. 'Sunshine'	USA, Louisiana	N+
S^{A}	VET-MRD-0002	7751	V. zizanioides cv. 'Huffman'	USA, Florida (Louisiana)	N+
S^{A}	VET-RDH-0001	7767	V. zizanioides	Hong Kong (Thailand?)	N-
S^{A}	VET-RDH-0002	7768	V. zizanioides	Hong Kong (South China)	N-
S^{B}	VET-JG-23	7773	V. zizanioides	New Zealand, Northland	N
S^{B}	VET-EB-5997	7776	V. zizanioides	Netherlands Antilles, Bonaire (USA)	N
S^{B}	VET-JGN-0001	7777	V. zizanioides	USA, California	N+
S^{B}	VET-EAB-5262	7950	V. zizanioides	Philippines, Leyte	N
S^{B}	VET-CXH-0001	7952	V. zizanioides	China, Guiyang	N+
S^{B}	VET-JA-1-1	7954	V. zizanioides	Kenya, Nairobi, ICRAF	N
S^{B}	VET-JA-1-3	7956	V. zizanioides	Peru, Iquitos, ICRAF	N
S^{B}	VET-JA-1-4	7957	V. zizanioides	Peru, Iquitos, ICRAF	N

S^{B}	VET-JA-2-3	7960	V. zizanioides, Peru, Iquitos, ICRAF	N
S^{B}	VET-OSR-1-B	7961	V. zizanioides, Venezuela, Maracay (Carabobo)	N+
S^{B}*	VET-OSR-2	7962	V. zizanioides, Venezuela, Maracay (Bajo Seco)	N+
S^{B}	VET-HGR-01	7965	V. zizanioides, Colombia, Bogota	N+
S^{B}	VET-TS-F1	7967	V. zizanioides, Ethiopia, Filakit	N+
S^{B}	VET-TS-F2	7968	V. zizanioides, Ethiopia, Filakit	N+
S^{B}	VET-TS-F3	7969	V. zizanioides, Ethiopia, Filakit	N+
S^{B}	VET-TS-D1	7970	V. zizanioides, Ethiopia, Digitosh	N+
S^{B}	VET-TS-D2	7971	V. zizanioides, Ethiopia, Digitosh	N+
S^{B}	VET-TS-M1	7973	V. zizanioides, Ethiopia, Minikaba	N+
S^{B}	VET-TS-M2	7974	V. zizanioides, Ethiopia, Minikaba	N+
S^{B}	VET-TS-M3	7975	V. zizanioides, Ethiopia, Minikaba	N+
S^{B}	VET-HP-01	7986	V. zizanioides, Honduras, Zamorano	N
S^{B}	VET-HP-03	7988	V. zizanioides, USA, Florida (Louisiana)	N
S^{B}	VET-JMJS-VC1	8000	V. zizanioides, Mexico, Oaxaca (Vera Cruz)	N
S^{B}	VET-CED-0001	8002	V. zizanioides, Bolivia, Sucre (MASDAR germplasm?)	N
S^{B}	VET-DD-A1	8005	V. zizanioides, Ethiopia, Dilla, Gedio	N
S^{B}	VET-DD-B1	8006	V. zizanioides, Ethiopia, Dilla, Gedio	N
S^{B}	VET-DD-C1	8007	V. zizanioides, Ethiopia, Dilla, Gedio	N
S^{B}	VET-MB-01	8029	V. zizanioides, cv. 'Huffman', USA, Florida (Louisiana)	N+

Sunshine affinities: (S- = Sunshine pattern with one missing band, S+ = Sunshine pattern with one additional band).

$S+^{B}$	VET-IPA-MUIR-001	7989	V. zizanioides, Mozambique, Maputo	?
$S+^{B}$	VET-LW-0001	8048	V. zizanioides cv. 'Capitol', USA, Louisiana	N
$S-^{B}$*	VET-TGAVC-002	8051	V. zizanioides cv. 'AVC', Spain, Murcia (Am. Vet. Co., Texas)	N+

Sri Lanka (Chiapas) clone (SL):

SL^{B}*	VET-RN-001	7951	V. zizanioides, Sri Lanka, Colombo	N-
SL^{B}	VET-IMZ-AGA	7765	V. zizanioides, Malawi, Lilongwe	N-
SL^{B}	VET-SBR-VNN-96/2	7993	V. zizanioides, Sri Lanka, Kandy	N-
SL^{B}	VET-SBR-VNN-96/3	7994	V. zizanioides, Sri Lanka, Kandy	N-
SL^{B}	VET-SBR-VNN-96/4	7995	V. zizanioides, Sri Lanka, Kandy	N-
SL^{B}	VET-SBR-AN-96/2	7997	V. zizanioides, Sri Lanka, Kandy	N-
SL^{B}	VET-SBR-AN-96/4	7999	V. zizanioides, Sri Lanka, Kandy	N-
SL^{B}	VET-JMJS-CH1	8001	V. zizanioides, Mexico, Oaxaca (Chiapas)	N-

'Farmers Fodder' or 'Karnataka' (KM)

KM^{B} *	VET-TGKN-003	8052	V. zizanioides cv. 'Karnataka', Spain, Murcia (Malaysia, India)	N+

'Breeder' complex (G)

G^{B}*	VET-UCL-027	7981	V. zizanioides, India, Lucknow, CIMAP	L?
G^{B}*	VET-HP-02	7987	V. zizanioides, India, Uttar Pradesh, (USDA PI 554617, 'Carter')	YL

'Breeder' affinities: G+, G++ = with one(+) or two (++) extra band(s); G- = with a missing band.

$G+^{B}$	VET-JGN-0002	7778	V. zizanioides, USA, California (Philippines?)	YL?
$G++^{B}$*	VET-UCL-024	7980	V. zizanioides, India, Lucknow, CIMAP	?
$G+^{B}$*	VET-UCL-040	7982	V. zizanioides, India, Lucknow, CIMAP	?
$G-^{B}$*	VET-UCL-042	7983	V. zizanioides, India, Lucknow, CIMAP	?
$G+^{B}$*	VET-UCL-045	7984	V. zizanioides, India, Lucknow, CIMAP	?
$G+-^{B}$*	VET-UCL-M1	7985	V. zizanioides, India, Lucknow, CIMAP	?

Khus type of Northern India (Kh): (similar to Indian type I, cf. 7761)

Kh^{B}*	VET-SCRC-001	8035	V. zizanioides, USA, USDA (India)	YF+

'Ganges' complex (North India), loose group with considerable banding differences

I^{B}*	VET-BANG-B001	7723	V. zizanioides, Bangladesh	YF+

I^{B*}	VET-BANG-B002	7724 V. zizanioides, Bangladesh	YF+
I^{B*}	VET-BANG-B003	7725 V. zizanioides, Bangladesh	YF+
I^{B*}	VET-BANG-B004	7726 V. zizanioides, Bangladesh	YF+
I^{B*}	VET-USDA-U1	7735 V. zizanioides, India, Punjab, Simla (USDA PI 196257)	YF+
I^{B*}	VET-USDA-U2	7736 V. zizanioides, India, A-3225 (USDA PI 213903)	YF+
I^{B*}	VET-USDA-U3	7737 V. zizanioides, India (USDA PI 271633)	YF+
I^{B*}	VET-USDA-U4	7738 V. zizanioides, India, A-7026 (unverified) (USDA PI 302300)	YF+
I^{B*}	VET-USDA-U5	7739 V. zizanioides, India, NBPGR Hybrid 7 (USDA PI 538753)	YF+
I^{B*}	VET-USDA-U6	7740 V. zizanioides, India, BE-2668, NBPGR Hybrid 8 (USDA PI 538754)	YF+
I^{B*}	VET-USDA-U7	7741 V. zizanioides, India, BE-2668, NBPGR Hybrid 26 (USDA PI 538756)	YF+
I^{B}	VET-K-Dtp-1	7752 V. zizanioides, India, Orissa	YF+
I^{B}	VET-K-Pub-2	7753 V. zizanioides, India, Orissa	YF+
I^{B}	VET-K-Dnk-3	7754 V. zizanioides, India, Orissa	YF+
I^{B}	VET-K-Brk-8	7759 V. zizanioides, India, Orissa	YF+
I^{B}	VET-U-Blp-9	7760 V. zizanioides, India, Orissa	YF+
I^{B}	VET-U-Nlg-10	7761 V. zizanioides, India, Orissa	YF+
I^{B}	VET-U-Gsg-11	7762 V. zizanioides, India, Orissa	YF+
I^{B}	VET-U-Bdm-12	7763 V. zizanioides, India, Orissa	YF+
I^{B}	VET-CWDS-01	7764 V. zizanioides, Nepal, Kathmandu (lowlands) (low flowering)	?
I^{B*}	VET-UCL-005	7976 V. zizanioides, India, Lucknow, CIMAP	?
I^{B*}	VET-UCL-007	7978 V. zizanioides, India, Lucknow, CIMAP	?
I^{B*}	VET-BANG-B006-B	8037 V. zizanioides, Bangladesh	YF+

Ganges affinities: I- = Ganges type with one missing band

$I\text{-}^{B*}$	VET-BANG-B005-B	8036 V. zizanioides, Bangladesh	YF+
$I\text{-}^{B*}$	VET-TGSB-004	8053 V. zizanioides, cv. 'Sabah', Spain, Murcia (Malaysia)	N+
$I\text{-}^{B*}$	VET-TGSBB-005	8054 V. zizanioides, cv. 'Sabak Buntar', Spain, Murcia (Malaysia)	N+

Graft type (Gr)

Gr^{A*}	VET-PT-1C	7713 V. zizanioides cv. 'Graft', Australia, Queensland	YL+
Gr^{B}	VET-SBR-AN-96/1	7996 V. zizanioides, Sri Lanka, Kandy	?

Other V. zizanioides banding patterns (O):(various banding, each of which is different)

O^{B}	VET-SJC-2	7775 V. zizanioides, Malawi, Zomba	N+
O^{B*}	VET-TGML-001	8050 V. zizanioides, cv. 'Malaysia', Spain, Murcia (Malaysia)	N+
O^{B*}	VET-TGPB-006	8055 V. zizanioides, cv 'Parit Buntar', Spain, Murcia (Malaysia)	N+
O^{B}	VET-JM-PV1	8076 V. zizanioides, Costa Rica, Puerto Viejo	N?

Other Vetiveria species

V. elongata (R. Br.) Stapf (Eg): (very similar to one another)

Eg^{A*}	VET-PT-2A	7716 V. elongata, (narrow leaf), Australia, Northern Territory	YF-
Eg^{A*}	VET-PT-2B	7717 V. elongata, (broad leaf), Australia, Northern Territory	YF-

V. filipes (Benth.) C.E.Hubb. (Fp) (quite distinct, 7772 may be a different species or genus)

Fp^{B*}	VET-PT-2C	7718 V. filipes, Australia	YF-
Fp^{B*}	VET-FA-257810	7772 V. filipes, Australia, USDA PI 257810	YF+

V. nigritana (Benth.) Stapf (Ng): (very similar to one another)

Ng^{A}	VET-ISV-AGA	7766 V. nigritana, Malawi, Lilongwe (few seed)	YL?!

Ng^{B}	VET-SJC-1	7774	V. nigritana, Malawi, Zomba	YF+
Possible other Vetiveria/Chrysopogon species				
P^{A}	VET-RGG-PA-B	7720	Vetiveria sp.?, Panama, Western, site B (Costa Rica)	?
Vb^{B}	VET-BANG-B005	7727	Vetiveria sp.?, Bangladesh	YF+?
Vb^{B}	VET-BANG-B006	7728	Vetiveria sp.?, Bangladesh	YF+?
Other genera				
Chrysopogon Trin.				
Cf^{A}*	VET-CFP-219579	7769	C. fulvus (Spreng.)Chiov., Pakistan (USDA PI 219579)	YF
Cg^{A}*	VET-CGP-383762	7771	C. gryllus (L.) Trin., Turkey (USDA PI 383762)	YF
Ca^{B}*	VET-BANG-B007	8038	C. aciculatus (Retz.) Trin., Bangladesh	YF+
Cn^{B}*	VET-JVTH-ZN001	8040	Chrysopogon nemoralis (Balansa) Holttum (recv'd as Zizania nemoralis (Balansa) Camas), Thailand	Y?F?
Sorghum Moench.				
Sh^{A}*	VET-AW-01	8030	S. halepense (L.) Pers., USA, Texas	YF+
Sb^{A}*	VET-RPA-8030	8030	S. bicolor (L.) Moench., USA, Texas	YF+
Not tested: (NT = not tested; D = degraded DNA, see text)				
NT	VET-MJ-B1	7701	V. zizanioides, USA, North Carolina, fungus on seeds	
NT	VET-MJ-B2	7702	V. zizanioides, USA, North Carolina, fungus on seeds	
NT	VET-MJ-B3	7703	V. zizanioides, USA, North Carolina, fungus on seeds	
NT	VET-MJ-B4	7704	V. zizanioides, USA, North Carolina, fungus on seeds	
NT	VET-MJ-B5	7705	V. zizanioides, USA, North Carolina, fungus on seeds	
NT*	VET-USDA-F1	7734	V. filipes, Australia, USDA (PI 257810) (duplicate acc. under 7772)	YF+
NT	VET-K-Bdln-4	7755	Vetiveria sp., India, Orissa	YF+
NT	VET-K-Bdln-5	7756	Vetiveria sp., India, Orissa	YF+
NT	VET-K-Bdln-6	7757	Vetiveria sp., India, Orissa	YF+
NT	VET-K-Bdln-7	7758	Vetiveria sp., India, Orissa	YF+
NT	VET-JSC-0001	7953	V. zizanioides?, Cambodia (Australia)	?
NT	VET-JBH-1267	8039	C. schmidianus, Laos	?
DNA too degraded to use				
D*	VET-USDA-B6	7706	V. zizanioides, India, Punjab, Simla (USDA PI 196257)	YF
D*	VET-USDA-B7	7707	V. zizanioides, India, Punjab, Simla (USDA PI 196257)	YF
D*	VET-USDA-B8	7708	V. zizanioides, India, Punjab, Simla (USDA PI 196257)	YF
D*	VET-USDA-B9	7709	V. zizanioides, India, Punjab, Simla (USDA PI 196257)	YF
D*	VET-USDA-B10	7710	V. zizanioides, India, Punjab, Simla (USDA PI 196257)	YF
D*	VET-CFI-554618	7770	C. fulvus (Sprengel) Chiov., India (USDA PI 554618)	YF
D	VET-EAB-5261	7949	V. zizanioides, Philippines, Leyte	?
D	VET-JA-1-2	7955	V. zizanioides, Kenya, Nairobi, ICRAF	?
D	VET-JA-2-1	7958	V. zizanioides, Kenya, Nairobi, ICRAF	?
D	VET-JA-2-2	7959	V. zizanioides, Kenya, Nairobi, ICRAF	?
D	VET-NSC-01	7963	V. zizanioides, Cameroon, Mbingo Bamenda (Nigeria)	?
D	VET-NSC-02	7964	V. zizanioides, Cameroon, Maroua	?
D*	VET-HGR-02	7966	V. zizanioides, Colombia, Cundinamarca (flowering)	?
D	VET-TS-D3	7972	V. zizanioides, Ethiopia, Digitosh	N+
D*	VET-UCL-006	7977	V. zizanioides, India, CIMAP	?
D*	VET-UCL-008	7979	V. zizanioides, India, CIMAP	?
D	VET-SBR-VA-96/1	7990	V. zizanioides, Sri Lanka, Kandy	N?
D	VET-SBR-VH-96/1	7991	V. zizanioides, Sri Lanka, Kandy	N?
D	VET-SBR-VNN-96/1	7992	V. zizanioides, Sri Lanka, Kandy	N?
D	VET-SBR-AN-96/3	7998	V. zizanioides, Sri Lanka, Kandy	?
D	VET-BBG-001	8003	V. zizanioides, Ghana, Central	N+
D	VET-BBG-02	8004	V. fulvibarbus, Ghana, Central	N+

Table 2. Eighteen accessions of *Vetiveria*, *Chrysopogon* and *Sorghum* analysed using primers: 134, 184, 212, 218, 234, 239, 244, 250, 265, 268, 327, 346 and 347. Codes for fertility: N = no; Y = yes; F = fully; L = low

Code	Acc. #	Lab #	Material, origin, collector	Fertile?
SS	VET-RPA-7655	7655	V. zizanioides, Haiti	N
PB	VET-RGG-PA-B	7720	Vetiveria sp.?, Panama, Western site B (Costa Rica)	?
GR	VET-PT-1C	7713	V. zizanioides cv. 'Graft', Australia, Queensland	YL
EN	VET-PT-2A	7716	V. elongata (R. Br.) Stapf (narrow leaf), Australia	YF
EB	VET-PT-2B	7717	V. elongata (R. Br.) Stapf (broad leaf), Australia	YF
FP	VET-PT-2C	7718	V. filipes (Benth.) C.E.Hubb., Australia	YF
BG	VET-BANG-B001	7723	V. zizanioides, Bangladesh	YF
InP	VET-USDA-196257	7735	V. zizanioides, Simla, Punjab, India, USDA PI 196257	YF
In1	VET-K-Dtp-1	7752	V. zizanioides, Orissa, India	YF
In2	VET-K-Pnb-2	7753	V. zizanioides, Orissa, India	YF
In8	VET-K-Brk-8	7759	V. zizanioides, Orissa, India	YF
In10	VET-U-Nlg-10	7761	V. zizanioides, Orissa, India	YF
NP	VET-CWDS-01	7764	V. zizanioides, Kathmandu, Nepal (lowlands)	?
NG	VET-ISV-AGA-	7766	V. nigritana (Benth.) Stapf, Lilongwe, Malawi, Africa	YL?
CF	VET-CFP-219579	7769	Chrysopogon fulvus (Spreng.) Chiov., Pakistan, USDA PI 219579	YF
CG	VET-CGP-383762	7771	Chrysopogon gryllus (L.) Trin., Turkey, USDA PI 383762	YF
SH	VET-AW-01	8030	Sorghum halepense (L.) Pers. Texas, USA, commercial	YF
SB	VET-RPA-8031	8031	Sorghum bicolor (L.) Moench., Texas, USA, commercial	YF

Table 3. Germplasm of high priority for maintenance and evaluation

Type	Accession #	Lab #	Species Source	Fertile?
S[A]	VET-PT-1A	7711	V. zizanioides cv. 'Monto', Australia, Queensland	N+
S[A]	VET-MR-VAL1	7722	V. zizanioides cv. 'Vallonia', South Africa	N
S[A]	VET-GVB-001	7742	V. zizanioides cv. 'Boucard', USA	N+
S[B]	VET-MRL-001	7749	V. zizanioides cv. 'Sunshine', USA. Louisiana	N
S[B]	VET-MB-01	8029	V. zizanioides cv. 'Huffman', USA, Florida	N+
S[B]	VET-OSR-1-B	7961	V. zizanioides, Venezuela, Maracay (Carabobo)	N+
S+[B]	VET-IPA-MUIR-001	7989	V. zizanioides, Mozambique, Maputo	?
S+[B]	VET-LW-0001	8048	V. zizanioides cv. 'Capitol', USA, Louisiana	N
S-[B]	VET-TGAVC-002	8051	V. zizanioides cv. 'AVC', Spain, Murcia (Am. Vet. Co., Texas)	N+
SL[B]	VET-IMZ-AGA	7765	V. zizanioides, Malawi, Lilongwe	?!
SL[B]	VET-RN-001	7951	V. zizanioides, Sri Lanka, Colombo	N+?
SL[B]	VET-JMJS-CH1	8001	V. zizanioides, Mexico, Oaxaca (Chiapas)	N+?
CR[B]	VET-JM-PV1	8076	V. zizanioides? Costa Rica, Puerto Viejo	N?
Gr[A]	VET-PT-1C	7713	V. zizanioides cv. 'Graft', Australia, Queensland	YL+
Gr[B]	VET-SBR-AN-96/1	7996	V. zizanioides, Sri Lanka, Kandy	?
G+[B]	VET-JGN-0002	7778	V. zizanioides, USA, California (Philippines?)	YL?
KM[B]	VET-TGKN-003	8052	V. zizanioides, cv. 'Karnataka', Spain, Murcia (Malaysia)	N+
G[B]	VET-HP-02	7987	V. zizanioides, India, Uttar Pradesh, (USDA PI 554617, 'Carter')	YL+
P[A]	VET-RGG-PA-B	7720	Vetiveria sp.?, Panama, Western, site B (Costa Rica)	?
O[B]	VET-SJC-2	7775	V. zizanioides, Malawi, Zomba (few seed heads)	?
O[B]	VET-TGML-001	8050	V. zizanioides, cv. 'Malaysia', Spain, Murcia (Malaysia)	N+
I-[B]*	VET-TGSB-004	8053	V. zizanioides, cv. 'Sabah', Spain, Murcia	

			(Malaysia)	?
I-B*	VET-TGSBB-005	8054	V. zizanioides, cv. 'Sabak Buntar', Spain, Murcia (Malaysia)	?
O[B]	VET-TGPB-006	8055	V. zizanioides, cv. 'Parit Buntar', Spain, Murcia (Malaysia)	N+

Table 4. Comparison of the DNA obtained from leaves ground in CTAB, ddwater or ethanol

	Quality of DNA from leaves ground in:		
material	dd water	CTAB	Ethanol
Fabaceae			
alfalfa, fresh	--	++	++
alfalfa, silica dried	--	++	++
Cruciferae			
broccoli, fresh	--	++	++
broccoli, silica dried	+	++	++
Cupressaceae			
juniper, fresh	+(-,24h)	++	++
juniper, silica dried	--	++	++
Chenopodiaceae			
spinach, fresh	--	++	++
spinach, silica dried	--	+	++
Poaceae (grasses)			
maize, fresh	--	++	++
maize, silica dried	-	-	++
sorghum, fresh	--	+	++
sorghum, silica dried	--	-	++
rice, fresh	--	--	++
rice, silica dried	--	-	++
vetiver, fresh	--	--	++
vetiver, silica dried	--	--	++
wheat, fresh	--	++	++
wheat, silica dried	--	+	++

NB: 1) The ddwater and ethanol grindings were further incubated in ddwater for 20 min., 37°C, before adding CTAB and incubating for 30 min., 60°C.

2) DNA quality: ++ = very good, molecular weight of 20-50kbp; + = good, MW of 20-50 kpb, but some degraded DNA on gel ranging down to 200-300 bp. - = poor, essentially no DNA of MW 20-50 kbp, DNA smeared from 6kbp to 200-300 bp; -- = degraded DNA, MW of only 200-300 bp.

Fig. 1. RAPD banding pattern for primer 268 for vetiver accessions. Lane 1 = pGEM markers, lane 2 = vetiver, Malawi. Lanes 3-27 have the 'Sunshine' pattern (see Table 1 for accessions used in lanes 3-27).

Fig. 2. Minimum spanning network of fourteen Vetiveria accessions, two Chrysopogon species (C. fulvus, Cf; C. gryllus, Cg) and two Sorghum species (S. bicolor, Sb; S. halepense, Sh) using 222 RAPD bands. Note that all the vetiver taxa cluster together and that the two Chrysopogon species cluster loosely, but enter thru links (dashed lines) to vetiver taxa. The Sorghum taxa cluster separately to form a separate group.

Fig. 3. Principal coordinate analysis of fourteen Vetiveria accessions (closed circles), two Chrysopogon species (C. fulvus, Cf; C. gryllus, Cg) and two Sorghum species (S. bicolor, Sb; S. halepense, Sh) using 222 RAPD bands. Also highlighted are vetiver from Haiti (SS, cv. 'Sunshine'), Nepal(NP), Panama (PB), V. filipes from Australia (FP), two forms of V. elongata from Australia (narrow leafed EN, broad leafed EB), V. fililpes, Australia (FA) and putative V. nigritana from Malawi (NG). The unlabeled OTUs in the lower left are V. zizanioides from the Ganges plain. The nearest neighbor similarities of the outlying taxa (Cf, Cg, Sb, Sh) to the central cluster are indicated by the dotted lines and the decimal numbers. The similarity between the two Chrysopogon species (Cf, Cg) is denoted by the .67 above the dashed line. See text for discussion.

Fig. 4. Principal coordinate analysis of fourteen Vetiveria OTUs using 197 RAPD bands. Open stars = vetiver from India; Closed star = vetiver from Bangladesh; Crossed circle = accession from Nepal; GR = vetiver cv. 'Graft', Australia; SS = cv. 'Sunshine' from Haiti; PB = vetiver from Panama (PB); FP = V. filipes from Australia; EN, EB = narrow and broad leafed forms of V. elongata from Australia; NG = putative V. nigritana from Malawi. The dotted lines indicate the most similar OTU to the outlying OTU, with the similarity denoted by the decimal numbers. See text for discussion.

Fig. 5. Principal coordinate analysis of nine _Vetiveria zizanioides_ OTUs using 197 RAPD bands. GR = cv. 'Graft', Australia; SS = cv. 'Sunshine' from Haiti; InP = India, Punjab (USDA PI 196257); In1, In2, In8 = India; NP = Nepal; BG = Bangladesh. The dotted lines indicate the most similar OTU to the outlying OTU, with the similarity denoted by the decimal numbers. See text for discussion.

Fig. 6. Minimum spanning network for 23 vetiver accessions based on 217 RAPD bands. See Table 4 of code identifications.

Fig. 7. Principal coordinate analysis of the 23 vetiver accessions. Note that accessions B4-B9 are all putative V. nemoralis from Thailand. See text for discussion. See Table 4 of code identifications.

Fig. 8. PCO of 16, non-seedy vetivers. Note the divergence of the Malaysian accession (ML) and that the Thailand vetivers (B1-3) cluster well with Sunshine (SS). See Table 4 of code identifications

Fig. 9. PCO of the 16, non-seedy vetivers mapped onto coordinates 1, 4, and 5. Notice the divergence of AV (American Vetiver Corp.) and KR (Karnataka, Malayasia) from the other vetivers. See Table 4 of code identifications

Fig. 10. Effects of grinding buffer on DNA quality. C = CTAB used in grinding, W = ddwater used in grinding, E = ethanol used in grinding. All materials were dried in silica gel, 72 h, °C before extraction. Lane 1 and 14: lambda/HindIII markers, Lanes 2-4: maize, lanes 5-7: wheat; lanes 8-10: vetiver; lanes 11-13: sorghum.