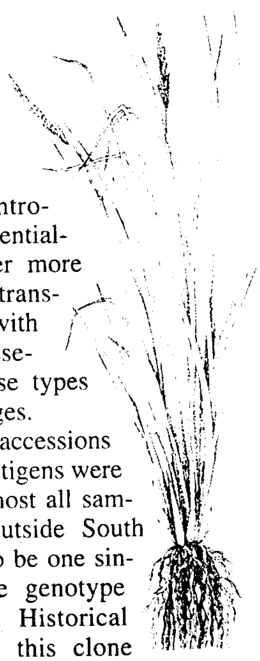
Lessons in Diversity: *DNA Sampling of the Pantropical Vetiver Grass Uncovers Genetic Uniformity in Erosion-Control Germplasm*



Vetiveria zizanioides

(Drawing courtesy World Bank)

by R.P. Adams and M.R. Dafforn

Recent research has again confirmed how new knowledge of genetic diversity can have direct and immediate benefits for the stakeholder community. In this case, DNA fingerprinting (Random Amplified Polymorphic DNAs [RAPDs]) has revealed high natural variability in vetiver grass while exposing unexpected genetic uniformity in cultivated materials.

Known for its fragrant roots and the essential oil it contains (CSIR, 1976), the aromatic grass vetiver (*Vetiveria zizanioides* (L.) Nash) has been grown for centuries in South Asia. Vetiver's economic value led to its introduction throughout the tropics more than 100 years ago. Historically, vetiver also has been used in contour hedges for erosion control in agriculture (Purseglove, 1972), but only recently has it gained widespread recognition from scientists, engineers, and development workers for its superb service in water conservation and flow management and for stabilizing field agriculture and earthen structures (NRC, 1993).

As a result of vetiver's impressive performance over the past decade, plantings have greatly expanded. Hedges are now used in more than 100 tropical countries for agriculture and civil engineering works. In almost every case, the foundation germplasm comes from traditional, in-country sources such as essential-oil plantations, herbal and botanical gardens, or other planted sites. Such local collecting has led to scores of uncharacterized accessions. Dealing with poorly defined diversity presented the challenge of systematizing relationships among individual hedge accessions.

Two Complexes of Vetiver

The botanical and agronomic literature distinguishes between two broad complexes of *Vetiveria zizanioides*:

1) **"North India"**: wild, fully fertile populations native across and beyond the Ganges Plain from Pakistan to at least Bangladesh, and

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2) **"South India"**: cultivated, nonfertile types traditionally grown for their essential oil in South India, Sri Lanka, and apparently Indochina, the Malay Archipelago and to the east, as well as perhaps Mauritius and Madagascar (see distribution map, p. 28, and box on this page).

> ***DNA fingerprinting has not only improved our knowledge about vetiver, but it has refined research priorities for the crop.***

Although the two types of vetiver can be distinguished chemically by experienced growers as well as in the field, no consistent botanical (floral) distinctions have been available to inform users of the type of vetiver they have acquired. To establish effective, permanent hedges, growers have preferred the cultivated-nonfertile vetivers over the fertile Ganges type (as well as other plants). Not only is this oil vetiver robust, dense, and erect, with deep, copious roots, wide adaptability, and high pest resistance, but it is also nonfertile. The latter is important for two reasons. First, all germplasm from one mother plant is clonal and, therefore, genetically identical; thus hedges are uniform and of known character. Second, nonfertile vetiver must be vegetatively propagated and does not spread from seed.

RAPDs Reveal Much Information

Two series of RAPDs yielded the following results (detailed analysis, expanding on work by Kresovich et al., 1994, is in Adams and Dafforn, 1997):

- All samples submitted as vetiver were genus *Vetiveria* (with one completely anomalous exception), indicating effective botanical and traditional identifications. All botanically identified species clustered as expected.
- High diversity was found in South Asia, especially among seedy types of *V. zizanioides*; this is consistent with sexually reproducing populations.
- No seedy types outside of their native range were found in hedge use, although fertile vetivers have long been present in Colombia, Haiti, and the United States, for example. This may reflect a previous preferential introduction of essential-oil clones over more conveniently transported seed, with subsequent reselection of these types for use in hedges.
- Multiple accessions of identical cultigens were identified. Almost all samples (88%) outside South Asia proved to be one single, nonfertile genotype ('Sunshine'). Historical records show this clone was distributed early this century or before, possibly via Mauritius. A second series of analyses detected seven other nonfertile clones; one common to Sri Lanka also is used in Malawi and Mexico.

DNA fingerprinting also served as a blind control on the physical features of germplasm. Growers submitted over 100

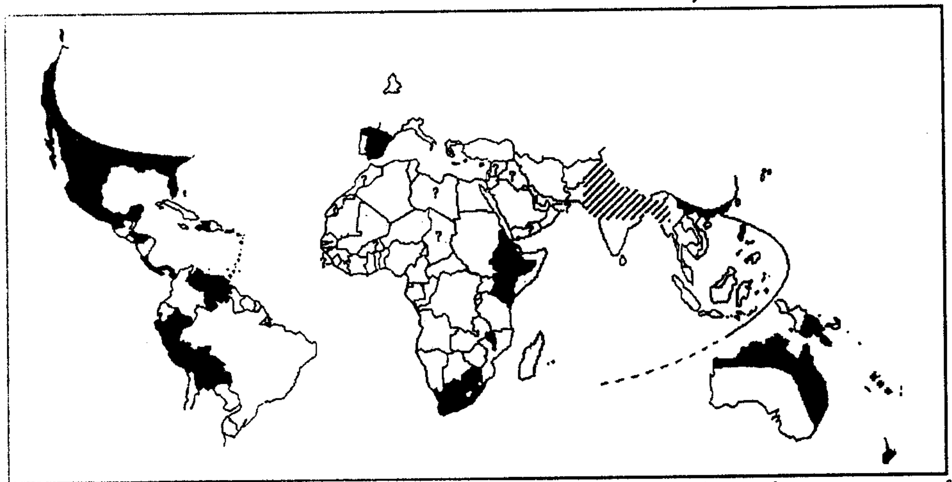
Describing Vetiver

Vetiver's long history of cultivation and widespread use has led scientists to apply many different terms to describe the plant. In the agronomic literature (most of which is Indian), the Ganges type is called "North India" vetiver; the nonfertile type is termed "South India" or "nonflowering." But the geographical names are misleading for both complexes extend beyond India. The horticultural term "nonflowering"—referring to the *relative* lack of flowering (generally less than 5%)—is also a misnomer, for all vetivers flower.

The oils of these two types differ chemically, and they are distinguished in commerce as "Khus Oil" (from the Ganges type) and "Oil of Vetiver" (from the essential-oil type). Until genetic and geographical characteristics separating these two genetic complexes are better understood, the terminology used to distinguish the two types includes the following:

- **North India**: "wild," "seedy," "fertile," "Ganges"
- **South India**: "cultivated," "nonseedy," "nonfertile,"* "essential-oil."

*Occasional caryopses (seeds) are formed in nonfertile vetivers but these are technically "nongerminative": in intensive testing none has produced viable seedlings. It seems likely the nonfertile vetivers are domesticates: as with potatoes and other root crops, selecting for improved root quantities and oil content allowed fertility to fall by the wayside (Dafforn, in press).



Distribution of Vetiveria zizanioides *is known throughout South Asia as well as in the warmer areas of Japan, China, New Zealand, Australia, France, Argentina, Chile, the United States, and Canada (British Columbia). The wild seedy type is native and largely restricted to the area around the Ganges Plain (shaded area).* (Map courtesy R.P. Adams)

field samples as either seeding or nonseeding. DNA results affirmed a distinction in every case. Confirmation of their *a priori* assessments has given users added confidence that their vetiver is nonfertile if it does not produce viable seed after flowering. Although this may seem self-evident, many species are indeterminate or set viable seed only intermittently. Still, much remains to be learned of vetiver's reproductive biology.

Concerns of Genetic Vulnerability Come with 'Sunshine'

As noted, most of the 60 samples from outside South Asia proved to be from a single clone, called 'Sunshine'* (named for a small town in Louisiana, USA, where USDA first purchased an heirloom plant in 1989.) This result does not seem to be a sampling artifact; 'Sunshine' apparently is a heritage cultigen that is found in many other countries.

The discovery that 'Sunshine' is in widespread use now allows research results from many countries (notably Australia, South Africa, and the United States) to be combined and directly compared. Researchers in multiple locations who unknowingly had been working with the same genotype have produced a synchronicity that has greatly reinforced our understanding of vetiver adaptability and behavior in a very short time.

The narrow genetic base currently utilized in agriculture, engineering, conservation, and long-term landscape rehabilitation is a modest but important concern. Clearly, the common use of the 'Sunshine' genotype outside of South Asia implies potential genetic vulnerability. This is common in many clonal crops such as potatoes ('Burbank'), bananas ('Cavendish'), or avocados ('Hass').

The common use of the 'Sunshine' genotype outside South Asia implies a high level of potential genetic vulnerability.

The 'Sunshine' genotype is remarkably robust and reliable and its use is encouraged. Nonetheless, scientists believe that expanding the number of available cultigens of hedge vetiver is prudent, and that diversifying the genotypes in field use may help forestall the pest and plague pressures inherent to monocropping. Vetiver users are fortunate in this regard. While there are no substitutes for such elite clones as 'Granny Smith' or 'Delicious' apples, several excellent genotypes of hedge vetiver are now known. Systematic collection and evaluation of vetiver are underway, and scientists hope that an assortment of nonfertile, phytosanitary materials will be available in time for the 2000 World Vetiver Congress in South Africa.

***'Sunshine' Vetiver:** RAPDs were used to examine 121 accessions of vetiver and related taxa from its region of origin and around the world. It appears that only one genotype, 'Sunshine,' accounts for almost all germplasm utilized outside South Asia. Curiously, no Sunshine types were detected from within the tropical Asian region of vetiver's early distribution. Additional analyses revealed that at least seven other non-fertile accessions are distinct genotypes. This germplasm diversity holds promise for reducing the genetic uniformity of what is now essentially a pantropical monoculture of an economically and environmentally important plant resource.

Conclusion

Clearly, DNA fingerprinting has not only improved our knowledge about and confidence in vetiver, but it has also refined research priorities for the crop. The focus has shifted now from documenting genomic variability to examining the degree of uniformity in exploited germplasm. Such advances illustrate once again how simple molecular diagnostics can prove of practical value with vetiver and other crops. Much remains to be learned of vetiver variability, and other nonfertile cultigens undoubtedly await identification. Additional studies are planned. A region of special focus is tropical Asia, where sampling has been low. Any readers with access to heritage vetivers are encouraged to contact the authors.

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References

Adams, R.P., and M.R. Dafforn. 1997. DNA fingerprints (RAPDs) of the pantropical grass vetiver, *Vetiveria zizanioides* (L.) Nash (Gramineae), reveal a single clone 'Sunshine' is widely utilized in erosion control. The Vetiver Network, Leesburg, VA. www.vetiver.org/resdna.htm.

Council on Scientific and Industrial Research (CSIR). 1976. Vetiveria. Pp. 451-457 in The Wealth of India, Vol. X. Publications & Information Director, CSIR, New Delhi.

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

Purseglove, J.W. 1972. Tropical Crops: Monocotyledons 1. John Wiley & Sons, Inc., New York.