

Genetic diversity of Vetiver clones (*Chrysopogon zizanioides* and *Chrysopogon nigritanus*) available in South Africa based on sequencing analyses

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Introduction

- Hydromulch = leading Vetiver suppliers in RSA
- The Vetiver supplied for rehabilitation is in the form of vegetative slips
- “Roley collection” = different Vetiver lines collected by Mr Roley Nöffke

The Roley Collection

CODE	COUNTRY	SPECIES	SUPPOSED ISOLATE NAME
#ACCVD0001	Congo, DRC	<i>C. zizanioides</i>	Kinshasa
#ACCVD0002	Madagascar (South)	<i>C. zizanioides</i>	
#ACCVD0003	Congo, DRC	<i>C. zizanioides</i>	Kinshasa
#ACCVD0004	Australia	<i>C. zizanioides</i>	Monto
#ACCVD0005	Mozambique	<i>C. nigritana</i>	
#ACCVD0006	Venezuela	<i>C. zizanioides</i>	Caracas
#ACCVD0007	South Africa	<i>C. zizanioides</i>	Rustenburg
#ACCVD0008	Ethiopia	<i>C. zizanioides</i>	
#ACCVD0009	Madagascar (North)	<i>C. zizanioides</i>	
#ACCVD0010	Congo, DRC	<i>C. nigritana</i>	Kinshasa
#ACCVD0011	Ghana		
#ACCVD0012	Ghana	<i>C. nigritana</i>	Buleng
#ACCVD0013	Ghana	<i>C. zizanioides</i>	Manga
#ACCVD0014	New Zealand	<i>C. zizanioides</i>	Mazda
#ACCVD0015	Kenya	<i>C. zizanioides</i>	
#ACCVD0016	Mozambique	<i>C. zizanioides</i>	Nampula
#ACCVD0017	Puerto Rico	<i>C. zizanioides</i>	Sunshine
#ACCVD0018	South Africa	<i>C. zizanioides</i>	Rustenburg
#ACCVD0019	Ghana	<i>C. zizanioides</i>	Manga

Taxonomical Overview

- *Chrysopogon* and *Vetiveria* considered as two distinct genera
- Roberty revised genera in 1960
- *Vetiveria* merged into *Chrysopogon*
- *Chrysopogon* tends to form a variety of ecotypes
- *C. nigritanus* and *C. zizanioides* almost undistinguishable on morphological level

Previous Genetic Studies

- RAPD analyses on Vetiver available in India done by Dong *et al* (2003)
- RAPD analyses on Vetiver available in Thailand done by Srifah, *et al*
- RAPD analyses on Vetiver available in Thailand done by Na Nakorn (1993)
- RAPD analyses on Vetiver done in the USA by Kresovich (1994)
- RAPD analyses on Vetiver done by Adams & Dafforn

Aims

- Determine phylogenetic position of Vetiver clones in RSA by sequencing
- Compare clones to other clones already sequenced

Material and Method

- Vetiver samples by Mr. Roley Nöffke, Hydromulch
- DNA extracted (Qiagen DNeasy Mini Plant isolation kit)
- Genes amplified via PCR
 - *ITS* region of 5.8S ribosomal gene
 - *ndhF* and *rbcl*

Table 2. PCR protocol

Primer Set	PCR Initial heat denaturation Temperature	PCR Initial heat denaturation duration	PCR Cycles	Annealing Temperature
<i>ITS</i>	95°C	2 minutes	30 cycles	58.0°C
<i>ndhF</i>	95°C	2 minutes	40 cycles	50.8°C
<i>rbcL</i>	95°C	2 minutes	35 cycles	63.5°C

- Products sequenced (Sanger method)
- Results analyzed (CLC workbench v6.0.2)
- Sequences aligned and edited

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- Results analyzed (CLC workbench v6.0.2)
- Sequences aligned and edited
- Construction of Phylogenetic trees (Neighbor-Joining trees)
 - Confirmed by Bootstrap analyses (1000 repetitions)
 - Software = ClustalX (v3.66)
 - Ref sequences and outgroups via BLAST

Results

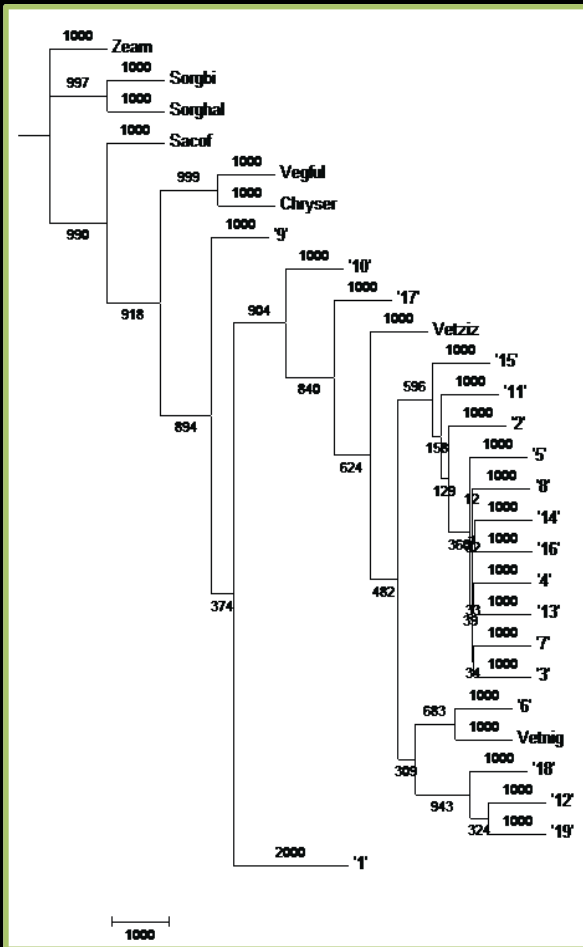


Figure 1. Neighbor-Joining tree of the 5.8 ribosomal and *ITS* gene.

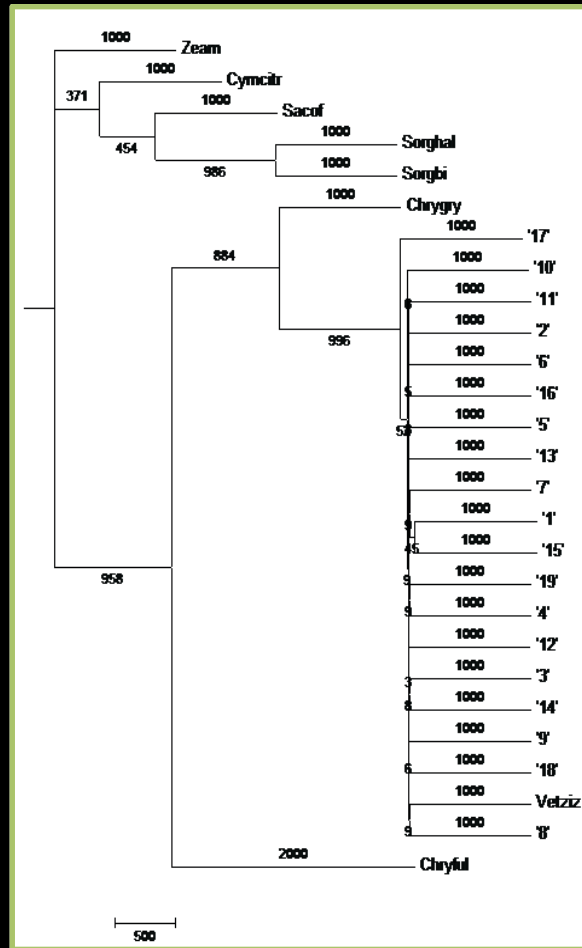


Figure 2. Neighbor-Joining tree of the *ndhF* chloroplast gene

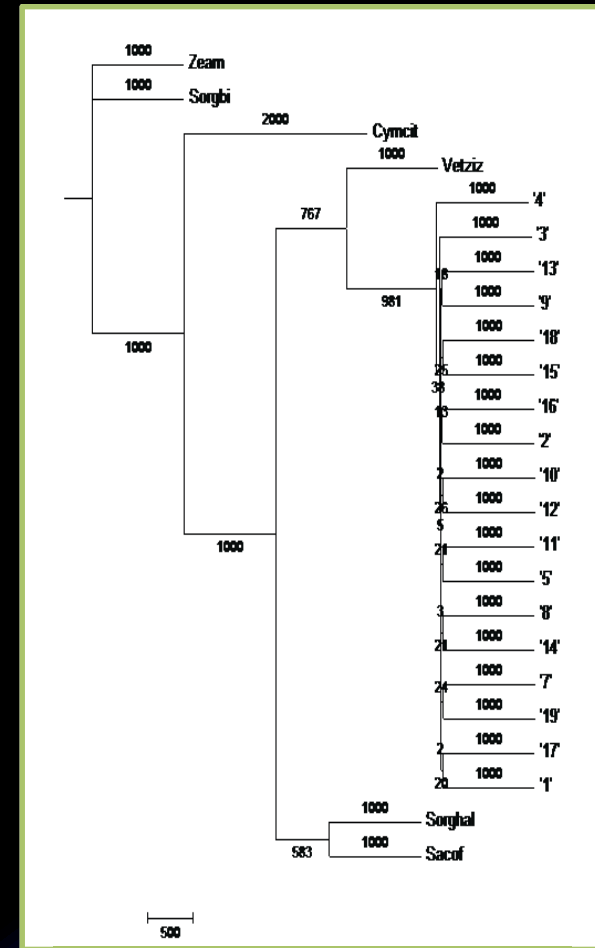


Figure 3. Neighbor-Joining tree of the *rbcL* chloroplast gene.

Conclusion

- *Zea, Sorghum, Saccharum* and *Chrysopogon* have a common ancestor
- Roley Collection clustered separately from *C. zizanioides* ref sequence
- Exception of *ndhF* gene
- *C. nigritanus* ref sequence clustered with Roley collection (*ITS* gene)
- Might indicate that Roley collection = more similar to *C. nigritanus*

- No statistically significant differences between isolates in Roley Collection
- Samples 4: Australia (*rbcL* gene), 9: Madagascar North (*ITS* gene), 10: DRC (*ITS* gene), and 17: Puerto Rico (*ITS* and *ndhF* gene), clustered separately from rest of Roley collection
- Basis for future genetic research

Recommendations

- Poliphase analysis
 - Biochemical analysis
 - Anatomical analysis
 - Morphological analysis
 - Symbiotic relationship analysis with micro-organisms

THE GREENING OF THE AUTOMOBILE



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"Doesn't it feel good to be taking care of our planet?"

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