

Symbiotic functioning and rhizobial biodiversity of Bambara groundnut (*Vigna subterranea* L. Verdc.)

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Bambara groundnut



Kersting's bean



INTRODUCTION

- Forms N₂ fixing symbioses with rhizobia from the family Rhizobiales.
- But with what?
- *Rhizobium*, *Bradyrhizobium*, *Ensifer*,
Azorhizobium or *Mesorhizobium*?

INTRODUCTION...

- Not much is known about N_2 fixation by Bambara groundnut in farmers' fields
- There is little information on the biodiversity of the rhizobia nodulating Bambara groundnut

OBJECTIVES

- To assess the relative dependency of Bambara groundnut on N_2 fixation
- To quantify and compare the amount of N-fixed by Bambara groundnut in farmers' fields in Mpumalanga Province
- To assess the biodiversity of microsymbionts nodulating Bambara groundnut
- To evaluate their symbiotic efficacy



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Table 1. Comparison of plant density, biomass yield and N content of Bambara groundnuts sampled from farmers' fields in 6 villages in the Mpumalanga Province, South Africa in 2009

Village	Plant density plant.m⁻²	Dry matter g.plant⁻¹	N content mg.plant⁻¹
Machipe	4.8c	40.2cd	1474.9b
Majakaneng	4.6c	50.7c	1452.8b
Dikgwale	4.7c	116.0a	3086.5a
Malekutu	7.6a	36.5d	1141.1c
Phameni	6.5b	31.8d	980.4d
Skhwahlane	6.4b	97.6b	3349.8a
<i>F- Statistics</i>	3.5***	21.6***	23.9***

Table 2. Comparison of $\delta^{15}\text{N}$, %Ndfa, N-fixed and Total soil N uptake of Bambara groundnuts sampled from farmers' fields in 6 villages in the Mpumalanga Province, South Africa in 2009.

Village	$\delta^{15}\text{N}$	Ndfa	N-fixed	Soil N
	‰	%	kg.ha⁻¹	
Machipe	1.00bc	73.3b	49.8d	22.6bc
Majakaneng	1.71b	79.6b	51.3d	17.3c
Dikgwale	1.26bc	64.8c	179.9a	39.5b
Malekutu	1.24bc	84.2ab	72.1bc	24.6bc
Phameni	-0.39c	87.5a	63.7c	14.3c
Skhwahlane	3.03a	76.9b	88.7b	93.0a
<i>F- Statistics</i>	24.8***	30.6***	1.9***	34.3***

Table 3. Estimates of N₂ fixation in food grain legumes in farmers' fields in South, Central and Northern Zambia. Means followed by dissimilar letters in a column for a region are significantly different at p≤ 0.05. Coefficient of variation ranged from 10 to 39 (Nyemba and Dakora 2010).

Species	Plant density no.m ⁻²	¹⁵ N shoot ‰	Ndfa %	N-fixed kg.ha ⁻¹
South				
Bambara	9.8b	2.2b	49ab	20.8b
Cowpea	11.4b	1.2b	60a	63.9a
Groundnut	22.8a	3.4a	27b	19.1b
Central				
Bambara	13.8ab	-0.2b	72a	33.5ab
Groundnut	20.0a	1.5a	31b	43.3a
Cowpea	10.2b	-0.2b	59a	35.4ab
Common bean	10.8b	-0.4b	55a	6.1c
North				
Bambara	14.8ab	0.5b	63a	67.6b
Groundnut	12.8b	1.6b	70a	78.7a
Common bean	24.2a	4.9a	3b	0.9c

Biodiversity of microsymbiont

Isolations

Authentication

16S rDNA sequencing

Table 4. A summary of morphological characteristics for the 287 isolates from Bambara groundnut nodules

Characteristics	No of isolates	% of isolates
Days to growth		
≤2	99	34.5
3-5	115	40.1
6-7	23	8.0
8-15	50	17.4
Alkali\acid producing		
Acidic	106	36.9
alkaline	181	63.1
Texture		
Buttery	153	53.3
Wet	134	46.7
-elastic	5	1.7
-non elastic	129	44.9
Appearance		
Opaque	213	74.2
Shiny	74	25.7
Shape		
Dome	248	86.4
Flat	39	13.5
Size		
≤2 mm	79	27.5
3-5 mm	88	30.7
6-8 mm	33	11.4
9-12 mm	21	7.3
13-15 mm	8	2.7
≥16 mm	58	20.2

AUTHENTICATION



Fig 1: Experimental setup for authenticating Bambara groundnut isolates in Leonard jars



Fig 2: Comparison of inoculated and uninoculated Bambara groundnut

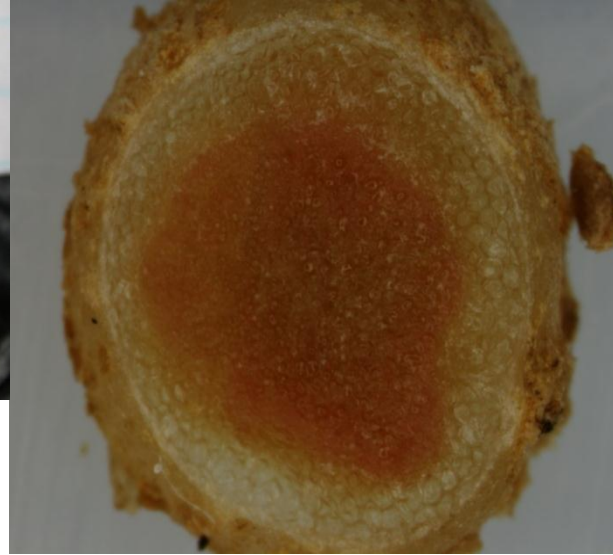


Fig 3: Effective and non-effective root-nodule of Bambara groundnut

Table 5. Symbiotic efficacy of authenticated fast-growing isolates from Bambara groundnut

Strains	Nodulation		Dry matter weight			
	Nodule no .plant ⁻¹	Nodule fresh weight	Shoot	Root	Whole plant	Shoot/root
(g.plant ⁻¹)						
Uninoculated	0.0f	0.0e	0.5d	0.1d	0.5d	3.0cd
Uninoculated	0.0f	0.0e	0.6c	0.2cd	0.8cd	3.5cd
TUTDigF2.2	0.0f	0.0e	0.2e	0.1d	0.3e	1.7e
TUTMacF4.2	0.0f	0.0e	0.2e	0.1d	0.3e	1.7e
TUTMajF1.3	24.0a	0.4c	0.3de	0.1d	0.4de	3.3cd
TUTMalF1.3	22.3ab	0.1e	0.2e	0.0e	0.3e	1.0f
TUTMalF1.6b	15.0b	0.4c	0.4d	0.3c	0.7cd	1.8e
TUTMalF2.4b	18.0b	0.5bc	0.7c	0.1d	0.9cd	6.2b
TUTMalF2.5b	4.3d	0.0e	0.3de	0.1d	0.4de	2.2de
TUTMalF2.8b	13.0c	0.3cd	1.3b	0.7a	1.9bc	4.3c
TUTMalF6.3a	23.3a	1.3a	3.6a	0.4b	4.1a	8.4a
TUTMalF6.7a	12.7c	0.3cd	0.5d	0.2cd	0.7cd	2.2de
TUTPhaF2.1b	11.7c	0.2d	1.1b	0.5ab	1.6bc	2.1de
TUTPhaF2.2b	16.3b	0.5bc	1.3b	0.7a	2.0b	2.1de
F- statistics	3.1***	8.9***	17.4***	3.7***	12.5***	3.2***

Table 6. Symbiotic efficacy of authenticated slow-growing isolates from Bambara groundnut

Strains	Nodulation		Dry matter weight			
	Nodule no Plant ⁻¹	Nodule fresh weight	Shoot	Root	Whole plant	Shoot/root
(g.plant ⁻¹)						
Uninoculated	0.0g	0.0f	0.5e	0.1d	0.6de	4.7cd
Uninoculated	0.0g	0.0f	0.5e	0.2c	0.8d	2.3e
TUTMalF1.1b	67.0b	1.0bc	1.2bc	0.2c	1.3c	7.5bc
TUTMalF1.2	74.0b	1.3ab	2.0a	0.3bc	2.3a	6.7c
TUTMalF1.6a	46.7d	0.6d	1.1c	0.4a	1.5bc	2.7e
TUTMalF2.4a	14.0f	0.5d	0.3f	0.1d	0.4e	2.3e
TUTMalF2.5a	88.0a	1.1b	1.8ab	0.4a	2.1ab	4.9cd
TUTMalF3.6a	81.0ab	0.6d	1.3bc	0.2c	1.5bc	6.5c
TUTMalF7.6a	57.3c	0.6d	1.6b	0.2c	1.8b	10.8ab
TUTMalF8.3a	68.3b	1.5ab	0.7d	0.2c	0.8d	4.5d
TUTMacF5.9	42.7d	1.2b	0.9cd	0.3b	1.2c	3.6d
TUTMacF5.10	86.0a	1.7a	0.5e	0.2c	0.7d	2.8e
TUTDigF2.1a	53.7c	0.8c	1.5b	0.3b	1.8b	4.8cd
TUTDigF2.8	31.0e	0.5d	0.4e	0.1d	0.5e	2.2e
TUTMajF1.1b	30.0e	1.1b	0.9cd	0.3b	1.2c	3.1de
TUTPhaF4.2b	45.7d	0.8c	1.8ab	0.2c	2.1ab	8.2b
F- statistics	4.3***	10.5***	17.3***	4.4***	14.8***	4.8***

Table 4: Relative effectiveness of Bambara groundnut isolates from five villages in Mpumalanga Province

Village	No of isolates	Highly effective	Moderately effective	Ineffective
			%	
Machipe	4	0	25	75
Majakaneng	2	0	0	100
Dikgwale	4	25	0	75
Malekutu	41	17	43	40
Phameni	12	33	50	17
Skhwahlane	2	0	50	50
Fast-grower	34	23	38	39
Slow-grower	31	19	48	33

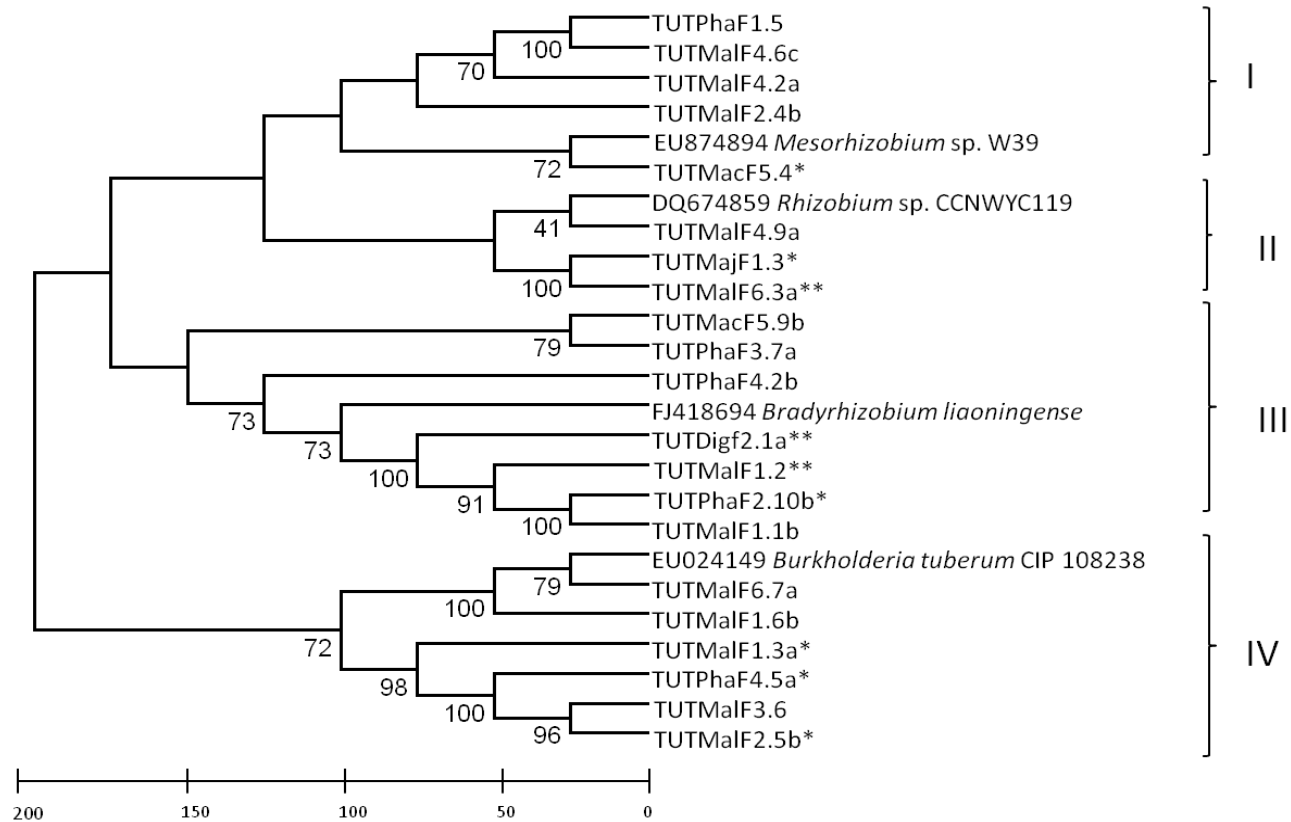


Figure 4: Phylogenetic relationships among 16S rDNA sequences of Bambara isolates

CONCLUSIONS

- 287 isolates showed differences in their growth, colony appearance, shape, texture and size
- Sequence analysis revealed that Bambara is nodulated by diverse microsymbionts that belong to both α - and β -rhizobia (subclasses of Proteobacteria) species
- Strains differed in their symbiotic effectiveness with *Bradyrhizobium* and *Rhizobium* species being highly effective

IMPLICATIONS

- It has the potential to be selected and bred for high N₂ fixation and improved grain yield
- Superior strains can be used for development of inoculants to improve yields



THANK YOU!!!!!!!!!!!!



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