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Science transforming food systems for a better future

Promiscuous nodulation of Bambara groundnut (*Vigna subterranea*) with different rhizobia species as a promise for sustainable smallholder agriculture and the bio-economy

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Brief Introduction

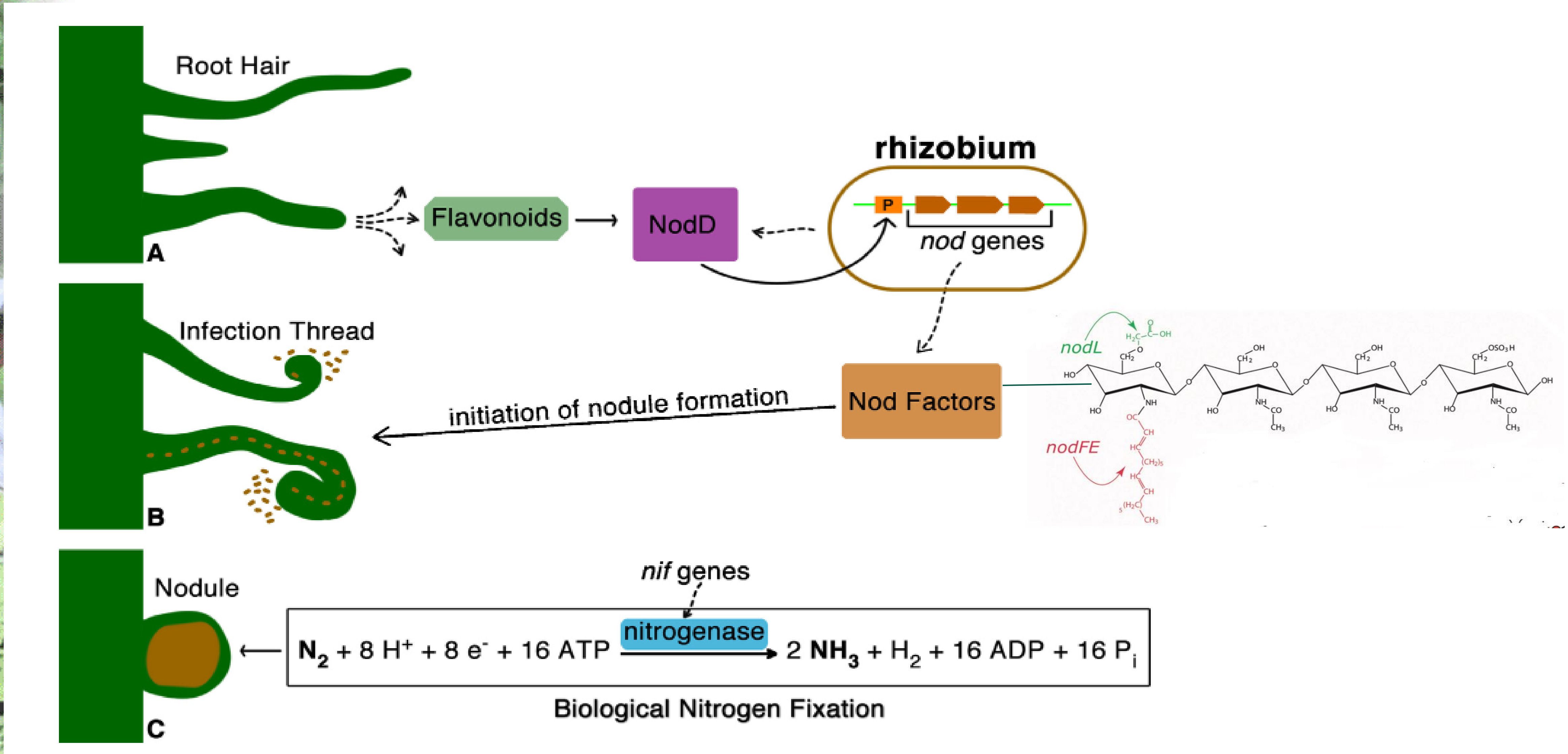
- **Drives its nitrogen (N) source through the process of *Biological Nitrogen Fixation* (BNF)**
- **Several genotypes (landraces) exist, hence nodulation of different genotypes by a given *Rhizobium* sp. is not uniform**
- **Very essential to identify (screen) bambara genotypes with superior nodulation and nitrogen fixation**
- **Contributions of such screening are important to:**
    - **i. Improve growth and yield of this underutilized legume**
    - **ii. Reduction of the application of inorganic fertilizers**
- **No information on the cultivar-rhizobia strain compatibility (symbiotic properties) of bambara in South Africa**

Vigna subterranea **(L.) Verdc (Bambara)**

Bambara groundnut (*Vigna subterranea* (L.) Verdc seeds (top) and potted plants and small pale yellow flowers of the plant

Sources: i) Wikipedia ii) https://sustainablebioresources.com/plants/plant-families/fabaceae-leguminosae-2/vigna-subterranea/

Biological Nitrogen Fixation (BNF)

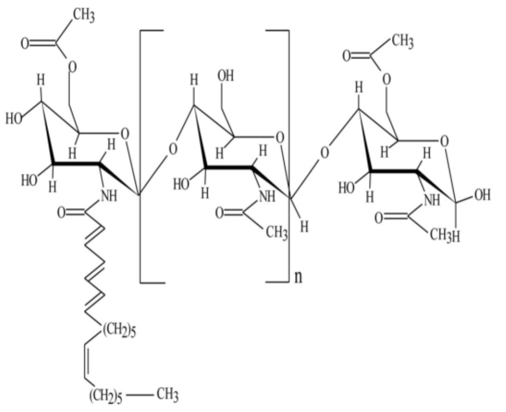
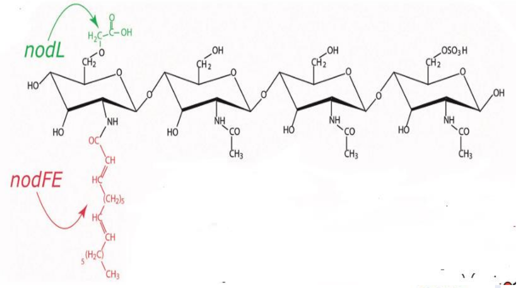
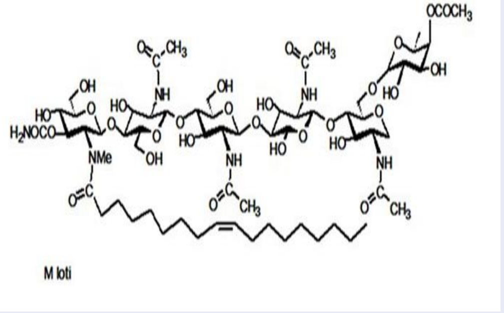


$$N_2 + 8\,H^+ + 8\,e^- + 16\,ATP \xrightarrow{\text{nitrogenase}} 2\,NH_3 + H_2 + 16\,ADP + 16\,P_i$$

Biological Nitrogen Fixation

Laranjo et al. 2014. *Microbiological Research 16*

Cross Inoculation Group

No	Cross Inoculation Group (CIG)	Microsymbionts	Rhizobial Nod-factors
1	Pea Group - *Pisum* spp. *Vicia* spp, *Lens culinaris, Vicia faba* (faba bean)	*Rhizobium leguminosarum* bv. viceae	
2	Bean Group - *Phaseolus vulgaris* (beans), *P. coccineus*	*Rhizobium leguminosarum* bv. phaseoli	
3	Clover group - *Trifolium* spp.	*R. leguminosarum* bv trifolii	
4	Alfalfa group - *Medicago, sativa, Medicago spp.*	*Sinorhizobium* (*Ensifer* spp.)	
5	Soybean group -*Glycine max*	*Bradyrhizobium japonicum*	
6	Chickpea group - Cicer arietinum	*Mesorhizobium* spp.	
7	Cow pea group - Pigeon pea, cowpea, peanut, mung bean, black gram, lima bean, bambara	Cow pea group - Pigeon pea, cowpea, peanut, mung bean, black gram, lima bean,	

Methodology I – Rhizobia spp. from the SARCC

Image: https://en.wikipedia.org/wiki/Genus_species

Desmodium uncinatum

Cyamopsis tetragonoloba

© B.Hacker

Arachis hypogeae

Glycine max

Phaseolus vulgaris

Five *Rhizobia* spp.

XBQ5 = SARCC-401

XBD2 = SARCC-388

XS34 = SARCC-578

WB1 = SARCC-304

UD5 = SARCC-715

Vigna subterranea (12 landraces/genotypes)

Photo: C.H. Bosch

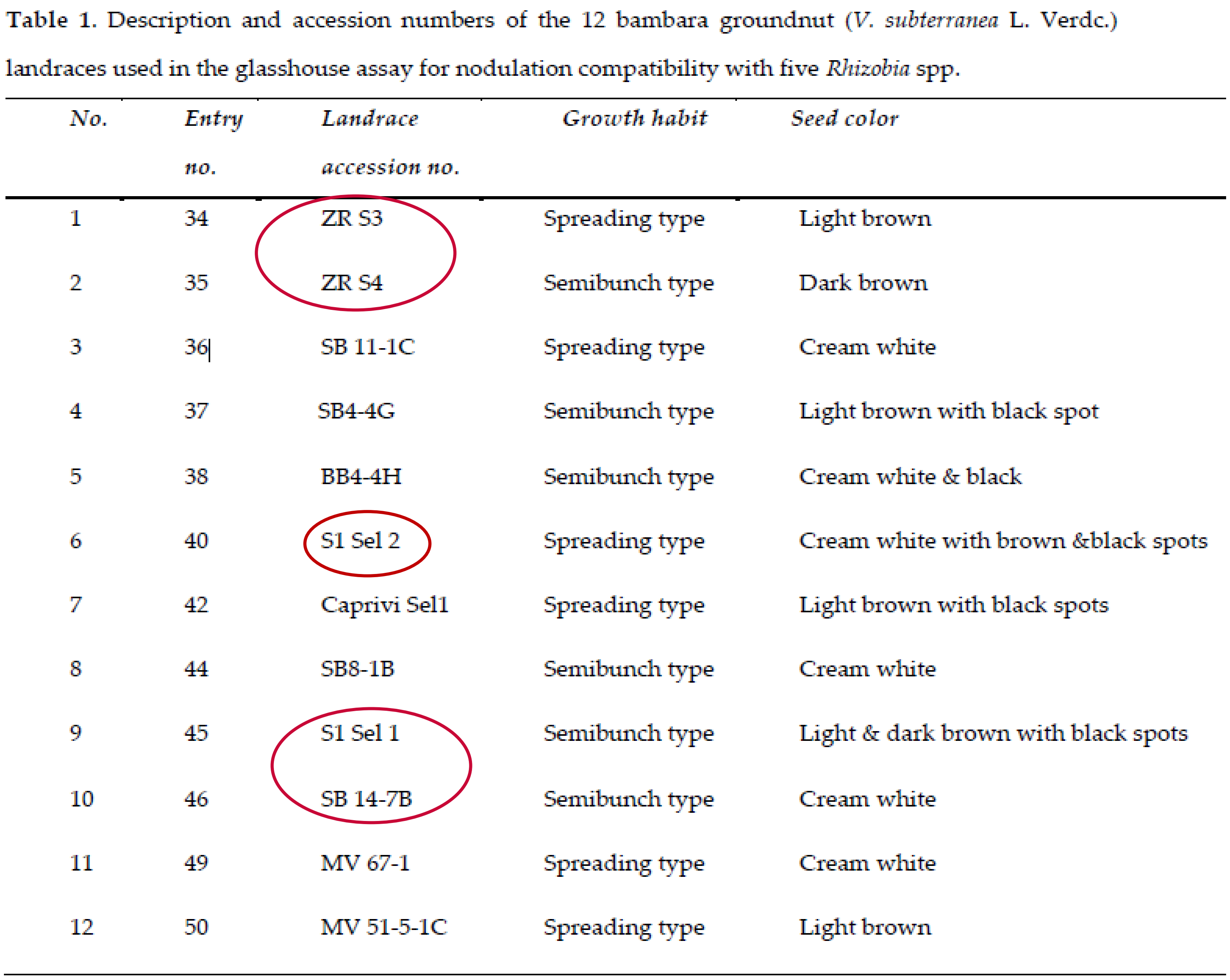


Table 1. Description and accession numbers of the 12 bambara groundnut (*V. subterranea* L. Verdc.) landraces used in the glasshouse assay for nodulation compatibility with five *Rhizobia* spp.

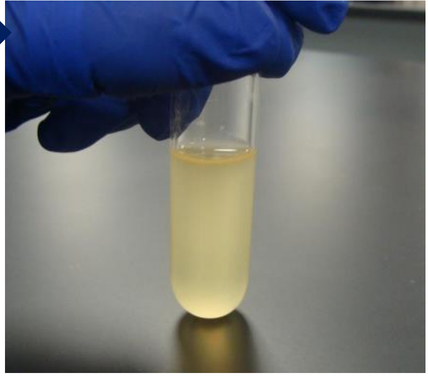
No.	*Entry no.*	*Landrace accession no.*	*Growth habit*	*Seed color*
1	34	ZR S3	Spreading type	Light brown
2	35	ZR S4	Semibunch type	Dark brown
3	36	SB 11-1C	Spreading type	Cream white
4	37	SB4-4G	Semibunch type	Light brown with black spot
5	38	BB4-4H	Semibunch type	Cream white & black
6	40	S1 Sel 2	Spreading type	Cream white with brown &black spots
7	42	Caprivi Sel1	Spreading type	Light brown with black spots
8	44	SB8-1B	Semibunch type	Cream white
9	45	S1 Sel 1	Semibunch type	Light & dark brown with black spots
10	46	SB 14-7B	Semibunch type	Cream white
11	49	MV 67-1	Spreading type	Cream white
12	50	MV 51-5-1C	Spreading type	Light brown

Inoculum preparation, planting and inoculation


XS34
Arachis hypogeae

Pure colonies of each rhizobium grown in liquid broth, concentration adjusted to ≥ 1 x 10^8 CFU/ml (= OD 1.0 -2.0)

OD_{600nm} = 1.0 -2.0



```
600.0nm -0.000Abs
NO.      Abs    K*Abs
2- 1   0.972    9.726
3- 1   1.824    18.24
4- 1   2.202    22.01
5- 1   1.962    19.62
6- 1   2.079    20.78
```

2 ml of each bacterial suspension per seed applied

5 bacteria x
12 rhizobia x
4 reps, split plot design
(Leonard Jar)

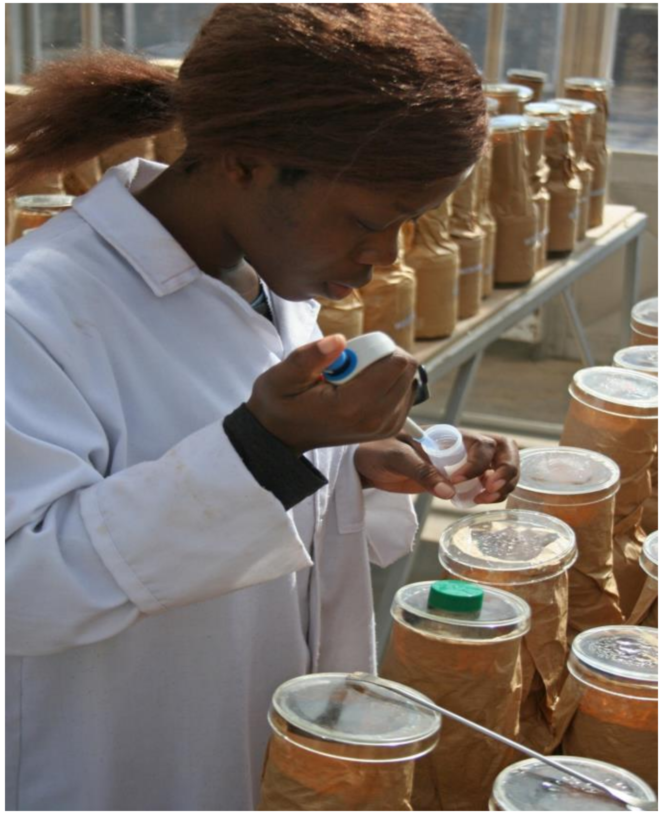
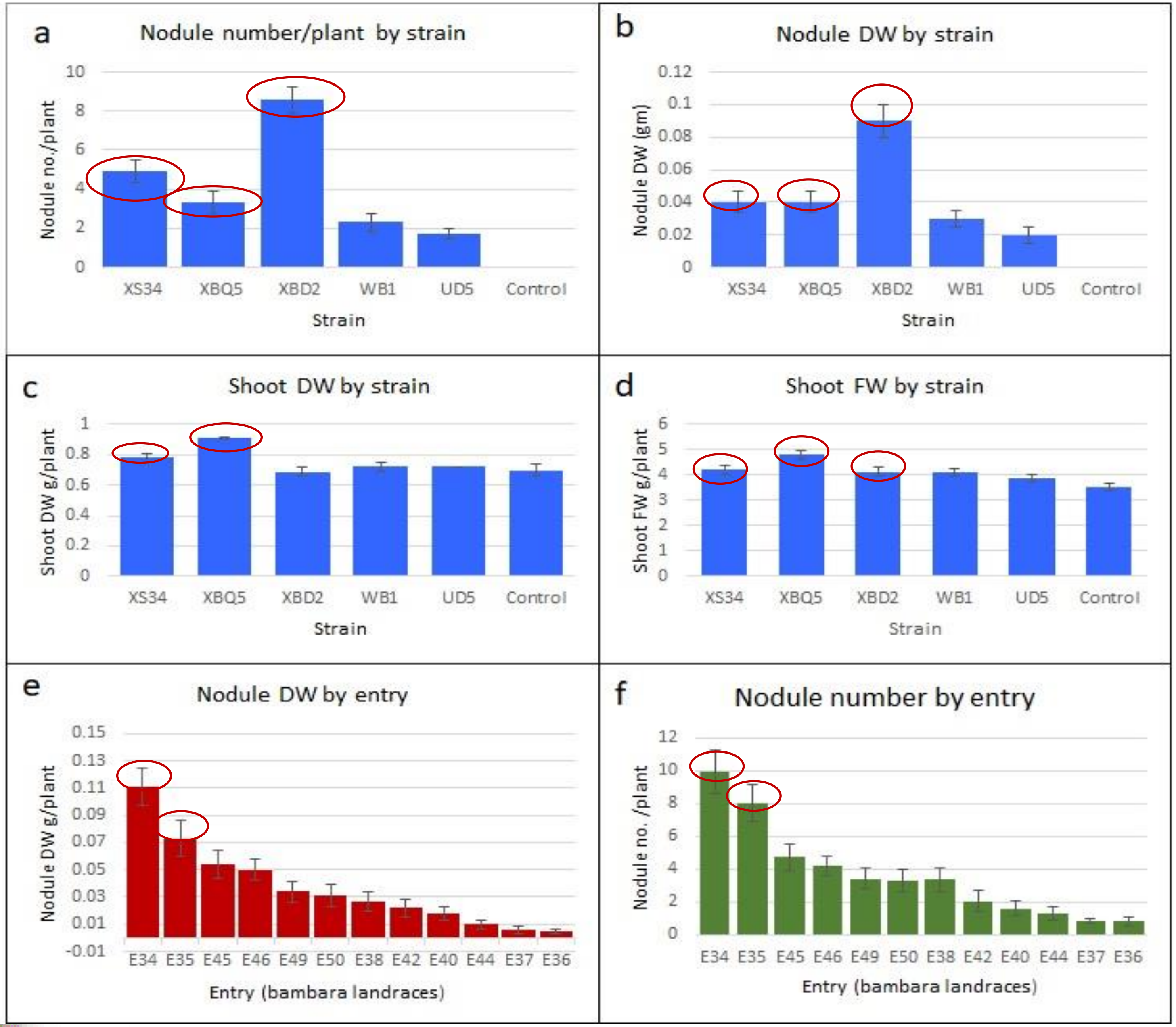
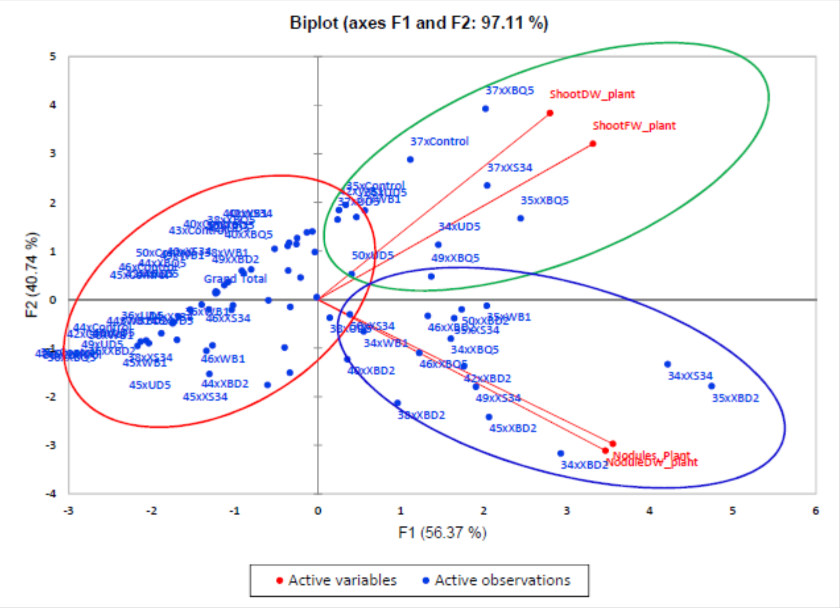


Photo source: This study

Results and Discussion

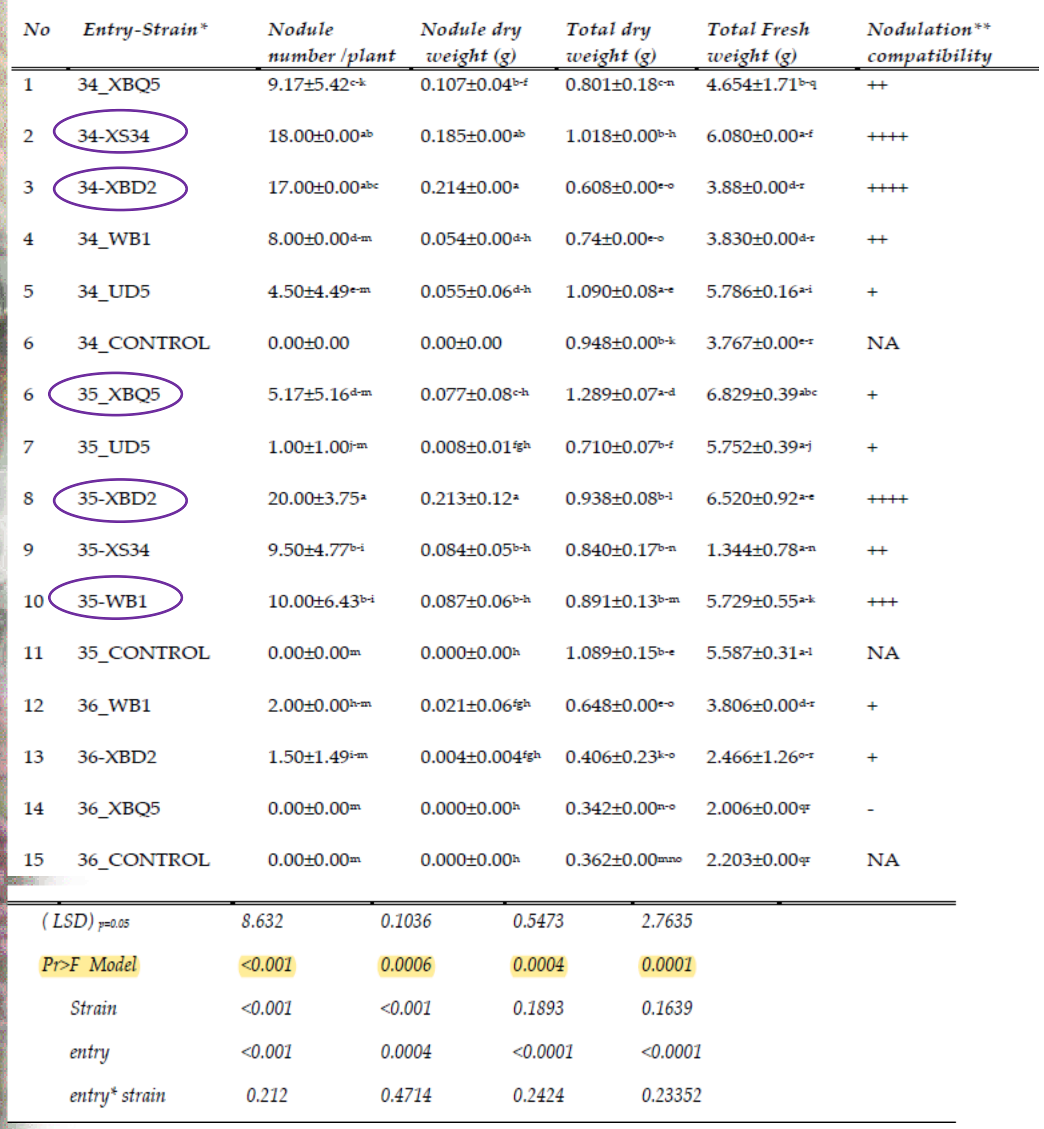
- ***Rhizobia*** **strains XBD2 & XS34 showed nodulation compatibility with 11/12 & 9/12 landraces of bambara respectively. While *Rhizobium* strain UD5 was the least compatible strain with all the 12 landraces**


a
Nodule number/plant by strain
Nodule no./plant
0
2
4
6
8
10
XS34
XBQ5
XBD2
WB1
UD5
Control
Strain
b
Nodule DW by strain
Nodule DW (gm)
0
0.02
0.04
0.06
0.08
0.1
0.12
XS34
XBQ5
XBD2
WB1
UD5
Control
Strain
c
Shoot DW by strain
Shoot DW g/plant
0
0.2
0.4
0.6
0.8
1
XS34
XBQ5
XBD2
WB1
UD5
Control
Strain
d
Shoot FW by strain
Shoot FW g/plant
0
1
2
3
4
5
6
XS34
XBQ5
XBD2
WB1
UD5
Control
Strain
e
Nodule DW by entry
Nodule DW g/plant
-0.01
0.01
0.03
0.05
0.07
0.09
0.11
0.13
0.15
E34
E35
E45
E46
E49
E50
E38
E42
E40
E44
E37
E36
Entry (bambara landraces)
f
Nodule number by entry
Nodule no. /plant
0
2
4
6
8
10
12
E34
E35
E45
E46
E49
E50
E38
E42
E40
E44
E37
E36
Entry (bambara landraces)


Biplot (axes F1 and F2: 97.11 %)
F2 (40.74 %)
F1 (56.37 %)
5
4
3
2
1
0
-1
-2
-3
-4
-3
-2
-1
0
1
2
3
4
5
6
ShootDW_plant
ShootFW_plant
Nodules_Plant
NoduleDW_plant
37xXBQ5
37xControl
37xXS34
35xXBQ5
35xControl
34xUD5
49xXBQ5
50xUD5
40xXBQ5
43xControl
49xXBD2
46xControl
44xXBQ5
Grand Total
46xXS34
35xWB1
50xXBD2
46xXBQ5
34xWB1
34xXBQ5
40xXBD2
42xXBD2
49xXS34
38xXBD2
45xXBD2
34xXBD2
34xXS34
35xXBD2
44xControl
49xUD5
46xWB1
45xWB1
45xUD5
44xXBD2
45xXS34
Active variables
Active observations

Results and Discussion

Table 2. Fisher's least significance difference (LSD)$_{p=0.05}$ test on the interaction between five rhizobia strains and 12 *Vigna subterranea* L. landrace collections in terms of nodulation compatibility, nodule dry weight and total plant biomass in a glasshouse inoculation trial.



No	*Entry-Strain**	*Nodule number /plant*	*Nodule dry weight (g)*	*Total dry weight (g)*	*Total Fresh weight (g)*	*Nodulation** compatibility*
1	34_XBQ5	9.17±5.42^{c-k}	0.107±0.04^{b-f}	0.801±0.18^{c-n}	4.654±1.71^{b-q}	++
2	34-XS34	18.00±0.00^{ab}	0.185±0.00^{ab}	1.018±0.00^{b-h}	6.080±0.00^{a-f}	++++
3	34-XBD2	17.00±0.00^{abc}	0.214±0.00^{a}	0.608±0.00^{e-o}	3.88±0.00^{d-r}	++++
4	34_WB1	8.00±0.00^{d-m}	0.054±0.00^{d-h}	0.74±0.00^{e-o}	3.830±0.00^{d-r}	++
5	34_UD5	4.50±4.49^{e-m}	0.055±0.06^{d-h}	1.090±0.08^{a-e}	5.786±0.16^{a-i}	+
6	34_CONTROL	0.00±0.00	0.00±0.00	0.948±0.00^{b-k}	3.767±0.00^{e-r}	NA
6	35_XBQ5	5.17±5.16^{d-m}	0.077±0.08^{c-h}	1.289±0.07^{a-d}	6.829±0.39^{abc}	+
7	35_UD5	1.00±1.00^{j-m}	0.008±0.01^{fgh}	0.710±0.07^{b-f}	5.752±0.39^{a-j}	+
8	35-XBD2	20.00±3.75^{a}	0.213±0.12^{a}	0.938±0.08^{b-l}	6.520±0.92^{a-e}	++++
9	35-XS34	9.50±4.77^{b-i}	0.084±0.05^{b-h}	0.840±0.17^{b-n}	1.344±0.78^{a-n}	++
10	35-WB1	10.00±6.43^{b-i}	0.087±0.06^{b-h}	0.891±0.13^{b-m}	5.729±0.55^{a-k}	+++
11	35_CONTROL	0.00±0.00^{m}	0.000±0.00^{h}	1.089±0.15^{b-e}	5.587±0.31^{a-l}	NA
12	36_WB1	2.00±0.00^{h-m}	0.021±0.06^{fgh}	0.648±0.00^{e-o}	3.806±0.00^{d-r}	+
13	36-XBD2	1.50±1.49^{i-m}	0.004±0.004^{fgh}	0.406±0.23^{k-o}	2.466±1.26^{o-r}	+
14	36_XBQ5	0.00±0.00^{m}	0.000±0.00^{h}	0.342±0.00^{n-o}	2.006±0.00^{qr}	-
15	36_CONTROL	0.00±0.00^{m}	0.000±0.00^{h}	0.362±0.00^{mno}	2.203±0.00^{qr}	NA
	(LSD) $_{p=0.05}$	*8.632*	*0.1036*	*0.5473*	*2.7635*	
	Pr>F Model	*<0.001*	*0.0006*	*0.0004*	*0.0001*	
	Strain	*<0.001*	*<0.001*	*0.1893*	*0.1639*	
	entry	*<0.001*	*0.0004*	*<0.0001*	*<0.0001*	
	entry strain*	*0.212*	*0.4714*	*0.2424*	*0.23352*	

Dendrogram

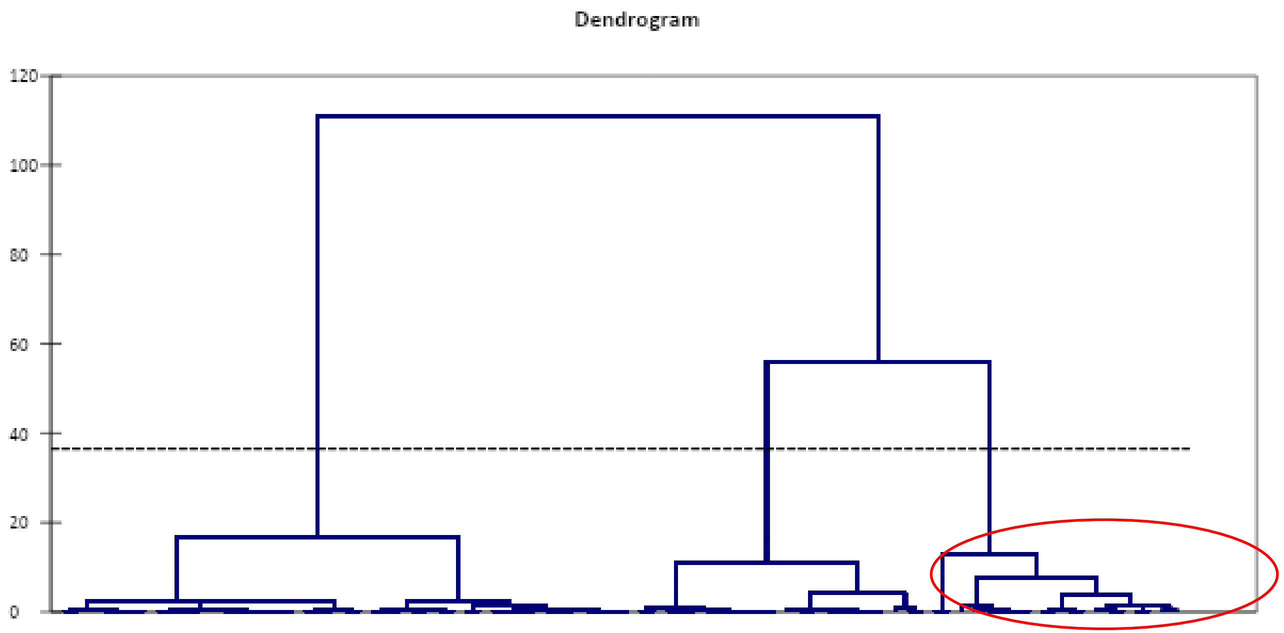


Table 3. Correlation matrix (Pearson (n-1)

Variables	**Nodules No/plant**	**Shoot FW**	**Shoot DW**	**Nodule DW**
Nodule No /plant	**1**	0.0254	0.089	0.973*
Shoot FW	0.254	**1**	0.897*	0.215
Shoot DW	0.089	0.897*	**1**	0.064
Nodule DW	0.973*	0.215	0.064	**1**

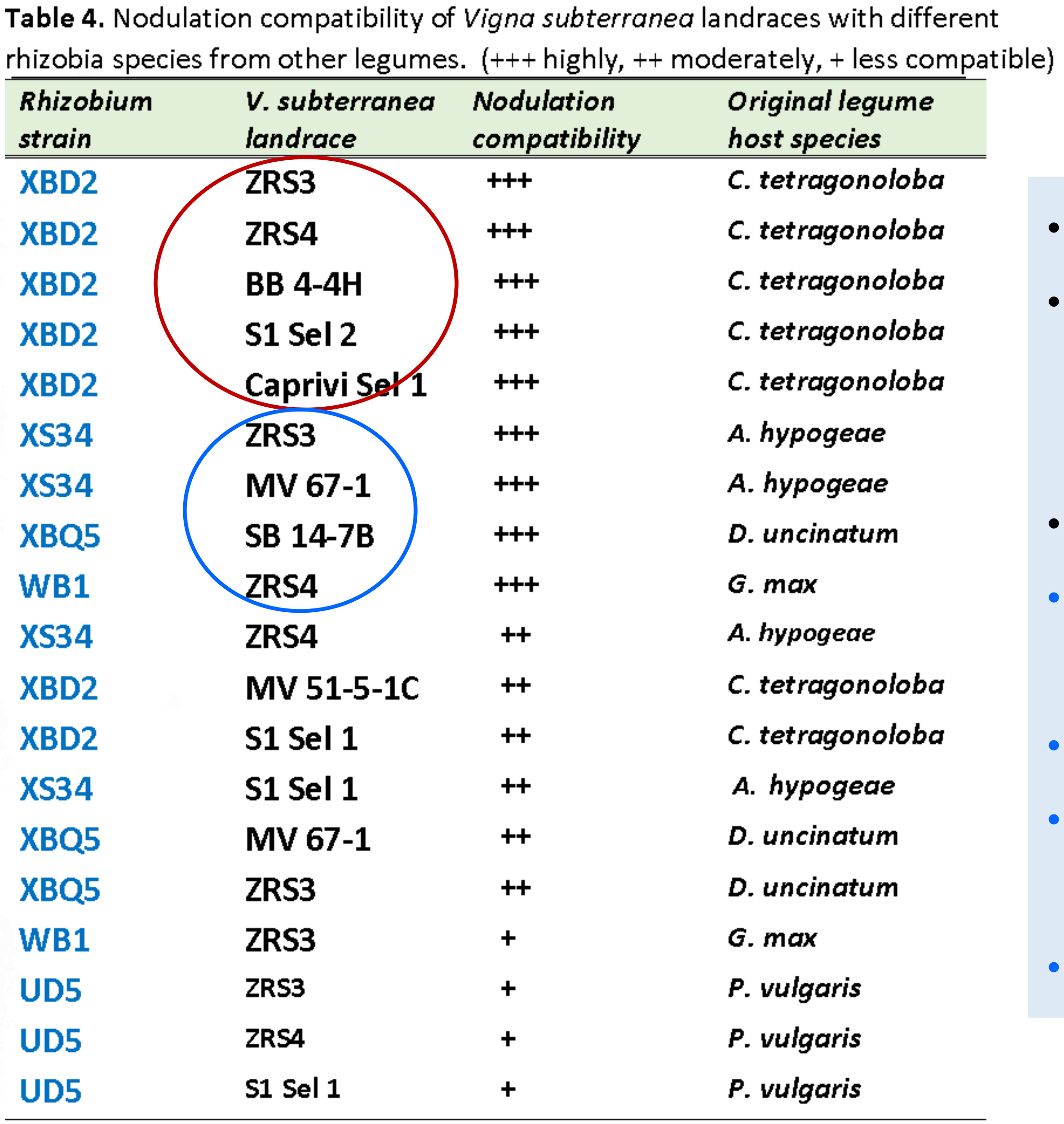


Table 4. Nodulation compatibility of *Vigna subterranea* landraces with different rhizobia species from other legumes. (+++ highly, ++ moderately, + less compatible)

Rhizobium strain	*V. subterranea landrace*	*Nodulation compatibility*	*Original legume host species*
XBD2	ZRS3	+++	*C. tetragonoloba*
XBD2	ZRS4	+++	*C. tetragonoloba*
XBD2	BB 4-4H	+++	*C. tetragonoloba*
XBD2	S1 Sel 2	+++	*C. tetragonoloba*
XBD2	Caprivi Sel 1	+++	*C. tetragonoloba*
XS34	ZRS3	+++	*A. hypogeae*
XS34	MV 67-1	+++	*A. hypogeae*
XBQ5	SB 14-7B	+++	*D. uncinatum*
WB1	ZRS4	+++	*G. max*
XS34	ZRS4	++	*A. hypogeae*
XBD2	MV 51-5-1C	++	*C. tetragonoloba*
XBD2	S1 Sel 1	++	*C. tetragonoloba*
XS34	S1 Sel 1	++	*A. hypogeae*
XBQ5	MV 67-1	++	*D. uncinatum*
XBQ5	ZRS3	++	*D. uncinatum*
WB1	ZRS3	+	*G. max*
UD5	ZRS3	+	*P. vulgaris*
UD5	ZRS4	+	*P. vulgaris*
UD5	S1 Sel 1	+	*P. vulgaris*

- ***≥ 10 nodules/plant formed by nine of the interactions***
- ***Rhizobium strain XBD2 showed nodulation compatibility with 11/12 of the landraces, forming 20 nodules/plant with landrace ZRS4 (entry 35)***
- ***High nodulation compatibility with five of the landraces***
- ***XS34 showed high nodulation compatibility with landraces ZRS3 , and MV 67-1***
- ***XBQ5 was highly compatible with landrace SB 14-7B***
- ***WB1 (unusual) was highly compatible with ZRS4 but incompatible with the rest of the landraces***
- ***UD5 was the least compatible strain***

Methodology II – Bacterial Identification

16S rRNA **and** ***recA*** **Genes Sequencing**

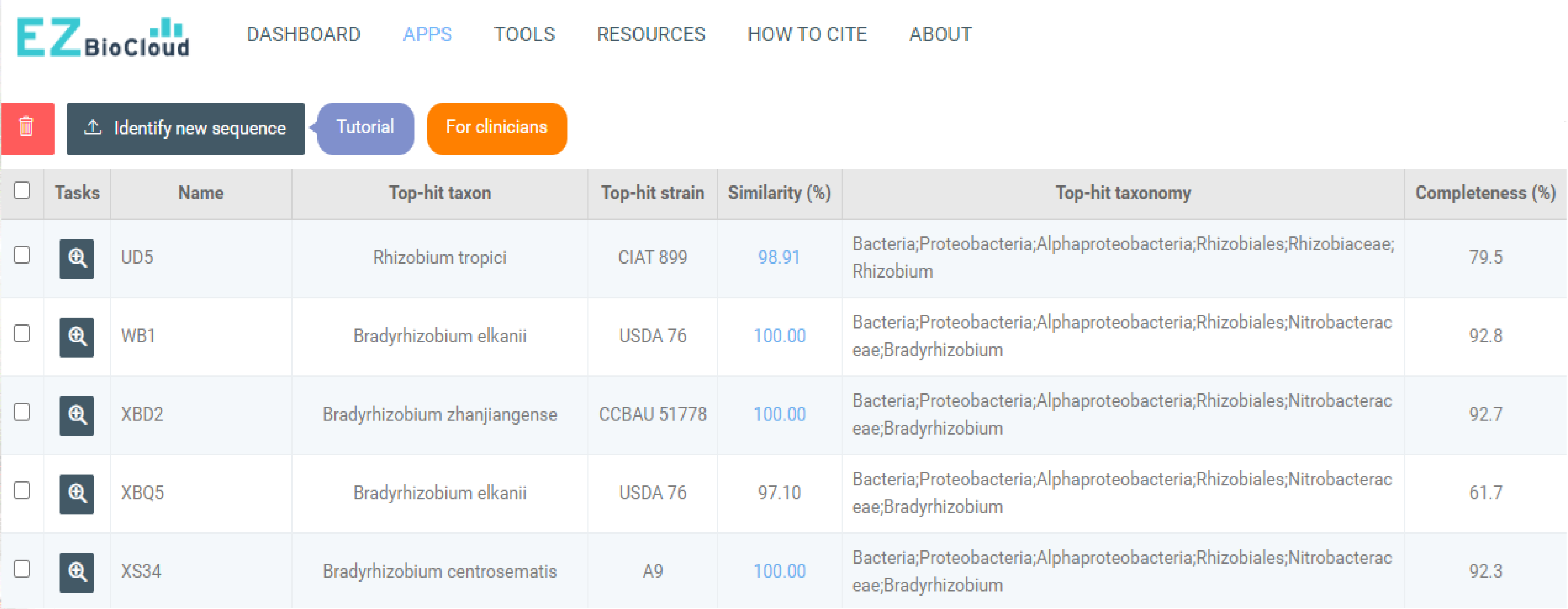
- **Rhizobia grown in Trypton Yeast Extract (TY) broth (100 ml), 28oC, 150 rpm, 24 – 48 h**
- **DNA extracted from 2 ml culture suspension using Wizard®Genomic DNA purification kit**
- **DNA quantified by measuring the absorbance of light at 260 nm**
- **PCR amplification: 50 µl total rxn (10µl Go taq flexi buffer, 1.5 mM MgCl2, 200 µl dNTPs, 1.25 U Go Taq polymerase, 0.8 µM each primer, 0.5 µg DNA template. (Sent to Inqaba Biotech, Pretoria)**

Target gene	Primer pair	PCR cycles	Reference
16S rRNA	fD1 (5’-AGAGTTTGATCCTGGCTCAG-3’) rD1 (5’-AAGGAGGTGATCCAGCC-3’)	95°C 3 min. 35 rounds of thermal cycling (94°C 1min, 72°C 2 min, 72°C 3 min.	Weisberg et. al 1991
recA	recA-63F (5’-ATCGAGCGGTCGTTCGGCAAGGG-3’) recA-504R (5’-TTGCGCAGCGCCTGGCTCAT-3’)	95°C, 5min. 30 rounds of thermal cycling (94°45s, 50°C, 60s and 74°C, 90s	Guant et al. 2001

Phylogenetic Analysis

- **Bioedit and Chromas Lite**
- **Consensus sequence BLASTn search on NCBI and EzBioCloud (https://www.ezbiocloud.net)**
- **Alignment with type strain using MAFFT online programme**
- **Neighbor Joining phylogenetic tree, Tamura-Nei statistical model, 1000 bootstrap reps**
- **MLSA tree – concatenated *recA* and *16S rRNA* sequences**
- **All sequences deposited at the NCBI GenBank library (accession numbers on Phylo trees)**

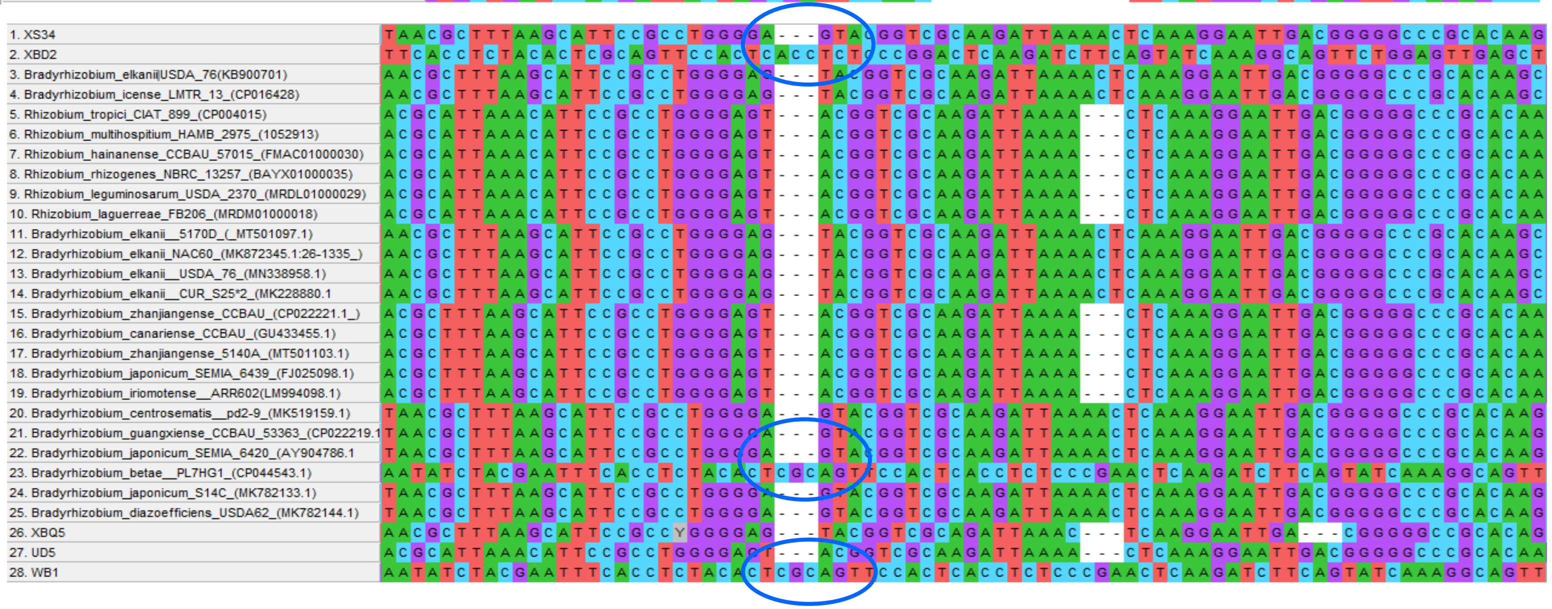
Result & Discussion: Sequence analysis and phylogeny



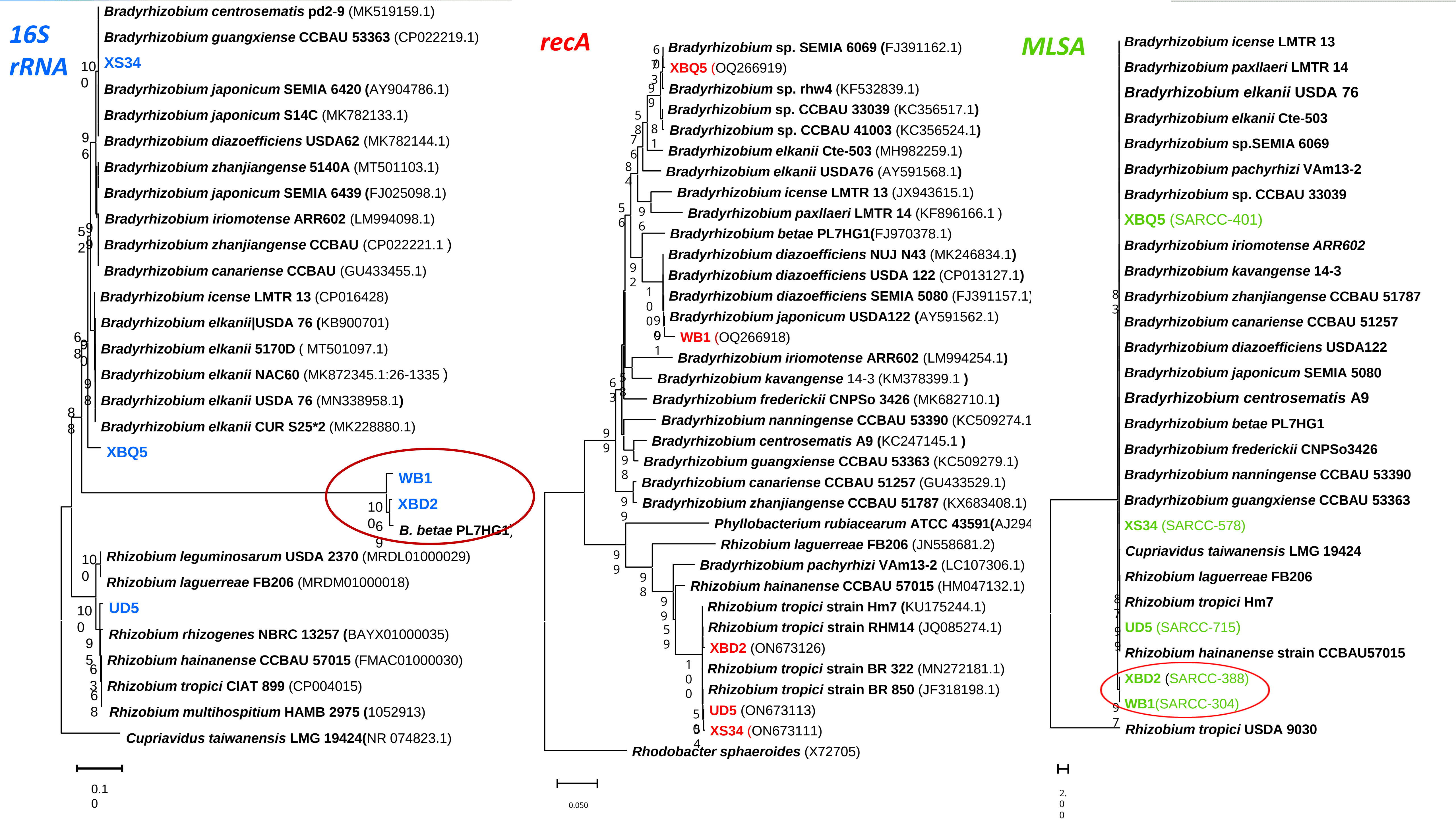
☐	Tasks	Name	Top-hit taxon	Top-hit strain	Similarity (%)	Top-hit taxonomy	Completeness (%)
☐	🔍	UD5	Rhizobium tropici	CIAT 899	98.91	Bacteria;Proteobacteria;Alphaproteobacteria;Rhizobiales;Rhizobiaceae;Rhizobium	79.5
☐	🔍	WB1	Bradyrhizobium elkanii	USDA 76	100.00	Bacteria;Proteobacteria;Alphaproteobacteria;Rhizobiales;Nitrobacteraceae;Bradyrhizobium	92.8
☐	🔍	XBD2	Bradyrhizobium zhanjiangense	CCBAU 51778	100.00	Bacteria;Proteobacteria;Alphaproteobacteria;Rhizobiales;Nitrobacteraceae;Bradyrhizobium	92.7
☐	🔍	XBQ5	Bradyrhizobium elkanii	USDA 76	97.10	Bacteria;Proteobacteria;Alphaproteobacteria;Rhizobiales;Nitrobacteraceae;Bradyrhizobium	61.7
☐	🔍	XS34	Bradyrhizobium centrosematis	A9	100.00	Bacteria;Proteobacteria;Alphaproteobacteria;Rhizobiales;Nitrobacteraceae;Bradyrhizobium	92.3

Consensus Sequence Alignment- MEGAX

1. XS34	CACAGGGAAACTTGTGCTAATACCGGATAAG------------CCCTTACGGGGAAAGATTTATCGCC
2. XBD2	TATTCACCGTGGCGTGCTGATCCACGATTACTAGCGATTCCAACTTCATGGGCTCGAGTTGCAGAGCC
3. Bradyrhizobium_elkaniiUSDA_76(KB900701)	TGAGGGAAACTTCAGCTAATACCGGATAAGC------------CCTTACGGGGAAAGATTTATCGCCG
4. Bradyrhizobium_icense_LMTR_13_(CP016428)	CCAGGGAAACTTGGGCTAATACCGGATAAGC------------CCTTACGGGGAAAGATTTATCGCCG
5. Rhizobium_tropici_CIAT_899_(CP004015)	ACGCAGGGAAACTTGTGCTAATACCGTATGT------------GTCCTTCGGGAGAAAGATTTATCGG
6. Rhizobium_multihospitium_HAMB_2975_(1052913)	ACGCAGGGAAACTTGTGCTAATACCGTATGT------------GTCCTTCGGGAGAAAGATTTATCGG
7. Rhizobium_hainanense_CCBAU_57015_(FMAC01000030)	ACGCAGGGAAACTTGTGCTAATACCGTATGT------------GTCCTTCGGGAGAAAGATTTATCGG
8. Rhizobium_rhizogenes_NBRC_13257_(BAYX01000035)	ACGCAGGGAAACTTGTGCTAATACCGTATGT------------GTCCTTCGGGAGAAAGATTTATCGG
9. Rhizobium_leguminosarum_USDA_2370_(MRDL01000029)	ACGCAGGGAAACTTGTGCTAATACCGTATGT------------GTCCTTCGGGAGAAAGATTTATCGG
10. Rhizobium_laguerreae_FB206_(MRDM01000018)	ACGCAGGGAAACTTGTGCTAATACCGTATGT------------GTCCTTCGGGAGAAAGATTTATCGG
11. Bradyrhizobium_elkanii__5170D_(_MT501097.1)	TGAGGGAAACTTCAGCTAATACCGGATAAGC------------CCTTACGGGGAAAGATTTATCGCCG
12. Bradyrhizobium_elkanii_NAC60_(MK872345.1:26-1335_)	TGAGGGAAACTTCAGCTAATACCGGATAAGC------------CCTTACGGGGAAAGATTTATCGCCG
13. Bradyrhizobium_elkanii__USDA_76_(MN338958.1)	TGAGGGAAACTTCAGCTAATACCGGATAAGC------------CCTTACGGGGAAAGATTTATCGCCG
14. Bradyrhizobium_elkanii__CUR_S25*2_(MK228880.1	TGAGGGAAACTTCAGCTAATACCGGATAAGC------------CCTTACGGGGAAAGATTTATCGCCG
15. Bradyrhizobium_zhanjiangense_CCBAU_(CP022221.1_)	ACACAGGGAAACTTGTGCTAATACCGGATAA------------GCCCTTACGGGGAAAGATTTATCGC
16. Bradyrhizobium_canariense_CCBAU_(GU433455.1)	ACACAGGGAAACTTGTGCTAATACCGGATAA------------GCCCTTACGGGGAAAGATTTATCGC
17. Bradyrhizobium_zhanjiangense_5140A_(MT501103.1)	ACACAGGGAAACTTGTGCTAATACCGGATAA------------GCCCTTACGGGGAAAGATTTATCGC
18. Bradyrhizobium_japonicum_SEMIA_6439_(FJ025098.1)	ACACAGGGAAACTTGTGCTAATACCGGATAA------------GCCCTTACGGGGAAAGATTTATCGC
19. Bradyrhizobium_iriomotense__ARR602(LM994098.1)	ACACAGGGAAACTTGTGCTAATACCGGATAA------------GCCCTTACGGGGAAAGATTTATCGC
20. Bradyrhizobium_centrosematis__pd2-9_(MK519159.1)	CACAGGGAAACTTGTGCTAATACCGGATAAG------------CCCTTACGGGGAAAGATTTATCGCC
21. Bradyrhizobium_guangxiense_CCBAU_53363_(CP022219.1	CACAGGGAAACTTGTGCTAATACCGGATAAG------------CCCTTACGGGGAAAGATTTATCGCC
22. Bradyrhizobium_japonicum_SEMIA_6420_(AY904786.1	CACAGGGAAACTTGTGCTAATACCGGATAAG------------CCCTTACGGGGAAAGATTTATCGCC
23. Bradyrhizobium_betae__PL7HG1_(CP044543.1)	TGCTGATCCACGATTACTAGCGATTCCAACT------------TCATGGGCTCGAGTTGCAGAGCCCA
24. Bradyrhizobium_japonicum_S14C_(MK782133.1)	CACAGGGAAACTTGTGCTAATACCGGATAAG------------CCCTTACGGGGAAAGATTTATCGCC
25. Bradyrhizobium_diazoefficiens_USDA62_(MK782144.1)	CACAGGGAAACTTGTGCTAATACCGGATAAG------------CCCTTACGGGGAAAGATTTATCGCC
26. XBQ5	--MGCC
27. UD5	ACGCAGGGAAACTTGTGCTAATACCGTATGT------------GTCCTTCGGGAGAAAGATTTATCGG
28. WB1	GTGCTGATCCACGATTACTAGCGATTCCAAC------------TTCATGGGCTCGAGTTGCAGAGCCC



1. XS34	TAACGCTTTAAGCATTCCGCCTGGGGA---GTACGGTCGCAAGATTAAAACTCAAAGGAATTGACGGGGGCCCGCACAAG
2. XBD2	TTCACCTCTACACTCGCAGTTCCACTCACCTCTCCCGGACTCAAGATCTTCAGTATCAAAGGCAGTTCTGGAGTTGAGCT
3. Bradyrhizobium_elkaniiUSDA_76(KB900701)	AACGCTTTAAGCATTCCGCCTGGGGAG---TACGGTCGCAAGATTAAAACTCAAAGGAATTGACGGGGGCCCGCACAAGC
4. Bradyrhizobium_icense_LMTR_13_(CP016428)	AACGCTTTAAGCATTCCGCCTGGGGAG---TACGGTCGCAAGATTAAAACTCAAAGGAATTGACGGGGGCCCGCACAAGC
5. Rhizobium_tropici_CIAT_899_(CP004015)	ACGCATTAAACATTCCGCCTGGGGAGT---ACGGTCGCAAGATTAAAA---CTCAAAGGAATTGACGGGGGCCCGCACAA
6. Rhizobium_multihospitium_HAMB_2975_(1052913)	ACGCATTAAACATTCCGCCTGGGGAGT---ACGGTCGCAAGATTAAAA---CTCAAAGGAATTGACGGGGGCCCGCACAA
7. Rhizobium_hainanense_CCBAU_57015_(FMAC01000030)	ACGCATTAAACATTCCGCCTGGGGAGT---ACGGTCGCAAGATTAAAA---CTCAAAGGAATTGACGGGGGCCCGCACAA
8. Rhizobium_rhizogenes_NBRC_13257_(BAYX01000035)	ACGCATTAAACATTCCGCCTGGGGAGT---ACGGTCGCAAGATTAAAA---CTCAAAGGAATTGACGGGGGCCCGCACAA
9. Rhizobium_leguminosarum_USDA_2370_(MRDL01000029)	ACGCATTAAACATTCCGCCTGGGGAGT---ACGGTCGCAAGATTAAAA---CTCAAAGGAATTGACGGGGGCCCGCACAA
10. Rhizobium_laguerreae_FB206_(MRDM01000018)	ACGCATTAAACATTCCGCCTGGGGAGT---ACGGTCGCAAGATTAAAA---CTCAAAGGAATTGACGGGGGCCCGCACAA
11. Bradyrhizobium_elkanii__5170D_(_MT501097.1)	AACGCTTTAAGCATTCCGCCTGGGGAG---TACGGTCGCAAGATTAAAACTCAAAGGAATTGACGGGGGCCCGCACAAGC
12. Bradyrhizobium_elkanii_NAC60_(MK872345.1:26-1335_)	AACGCTTTAAGCATTCCGCCTGGGGAG---TACGGTCGCAAGATTAAAACTCAAAGGAATTGACGGGGGCCCGCACAAGC
13. Bradyrhizobium_elkanii__USDA_76_(MN338958.1)	AACGCTTTAAGCATTCCGCCTGGGGAG---TACGGTCGCAAGATTAAAACTCAAAGGAATTGACGGGGGCCCGCACAAGC
14. Bradyrhizobium_elkanii__CUR_S25*2_(MK228880.1	AACGCTTTAAGCATTCCGCCTGGGGAG---TACGGTCGCAAGATTAAAACTCAAAGGAATTGACGGGGGCCCGCACAAGC
15. Bradyrhizobium_zhanjiangense_CCBAU_(CP022221.1_)	ACGCTTTAAGCATTCCGCCTGGGGAGT---ACGGTCGCAAGATTAAAA---CTCAAAGGAATTGACGGGGGCCCGCACAA
16. Bradyrhizobium_canariense_CCBAU_(GU433455.1)	ACGCTTTAAGCATTCCGCCTGGGGAGT---ACGGTCGCAAGATTAAAA---CTCAAAGGAATTGACGGGGGCCCGCACAA
17. Bradyrhizobium_zhanjiangense_5140A_(MT501103.1)	ACGCTTTAAGCATTCCGCCTGGGGAGT---ACGGTCGCAAGATTAAAA---CTCAAAGGAATTGACGGGGGCCCGCACAA
18. Bradyrhizobium_japonicum_SEMIA_6439_(FJ025098.1)	ACGCTTTAAGCATTCCGCCTGGGGAGT---ACGGTCGCAAGATTAAAA---CTCAAAGGAATTGACGGGGGCCCGCACAA
19. Bradyrhizobium_iriomotense__ARR602(LM994098.1)	ACGCTTTAAGCATTCCGCCTGGGGAGT---ACGGTCGCAAGATTAAAA---CTCAAAGGAATTGACGGGGGCCCGCACAA
20. Bradyrhizobium_centrosematis__pd2-9_(MK519159.1)	TAACGCTTTAAGCATTCCGCCTGGGGA---GTACGGTCGCAAGATTAAAACTCAAAGGAATTGACGGGGGCCCGCACAAG
21. Bradyrhizobium_guangxiense_CCBAU_53363_(CP022219.1	TAACGCTTTAAGCATTCCGCCTGGGGA---GTACGGTCGCAAGATTAAAACTCAAAGGAATTGACGGGGGCCCGCACAAG
22. Bradyrhizobium_japonicum_SEMIA_6420_(AY904786.1	TAACGCTTTAAGCATTCCGCCTGGGGA---GTACGGTCGCAAGATTAAAACTCAAAGGAATTGACGGGGGCCCGCACAAG
23. Bradyrhizobium_betae__PL7HG1_(CP044543.1)	AATATCTACGAATTTCACCTCTACACTCGCAGTTCCACTCACCTCTCCCGAACTCAAGATCTTCAGTATCAAAGGCAGTT
24. Bradyrhizobium_japonicum_S14C_(MK782133.1)	TAACGCTTTAAGCATTCCGCCTGGGGA---GTACGGTCGCAAGATTAAAACTCAAAGGAATTGACGGGGGCCCGCACAAG
25. Bradyrhizobium_diazoefficiens_USDA62_(MK782144.1)	TAACGCTTTAAGCATTCCGCCTGGGGA---GTACGGTCGCAAGATTAAAACTCAAAGGAATTGACGGGGGCCCGCACAAG
26. XBQ5	AACGCTTTAAGCATTCCGCCYGGGGAG---TACGGTCGCAGATTAAAC---TCAAGGAATTGA---CGGGGGCCGCACAG
27. UD5	ACGCATTAAACATTCCGCCTGGGGAGT---ACGGTCGCAAGATTAAAA---CTCAAAGGAATTGACGGGGGCCCGCACAA
28. WB1	AATATCTACGAATTTCACCTCTACACTCGCAGTTCCACTCACCTCTCCCGAACTCAAGATCTTCAGTATCAAAGGCAGTT


16S rRNA
Bradyrhizobium centrosematis pd2-9 (MK519159.1)
Bradyrhizobium guangxiense CCBAU 53363 (CP022219.1)
XS34
Bradyrhizobium japonicum SEMIA 6420 (AY904786.1)
Bradyrhizobium japonicum S14C (MK782133.1)
Bradyrhizobium diazoefficiens USDA62 (MK782144.1)
Bradyrhizobium zhanjiangense 5140A (MT501103.1)
Bradyrhizobium japonicum SEMIA 6439 (FJ025098.1)
Bradyrhizobium iriomotense ARR602 (LM994098.1)
Bradyrhizobium zhanjiangense CCBAU (CP022221.1)
Bradyrhizobium canariense CCBAU (GU433455.1)
Bradyrhizobium icense LMTR 13 (CP016428)
Bradyrhizobium elkanii|USDA 76 (KB900701)
Bradyrhizobium elkanii 5170D (MT501097.1)
Bradyrhizobium elkanii NAC60 (MK872345.1:26-1335)
Bradyrhizobium elkanii USDA 76 (MN338958.1)
Bradyrhizobium elkanii CUR S25*2 (MK228880.1)
XBQ5
WB1
XBD2
B. betae PL7HG1)
Rhizobium leguminosarum USDA 2370 (MRDL01000029)
Rhizobium laguerreae FB206 (MRDM01000018)
UD5
Rhizobium rhizogenes NBRC 13257 (BAYX01000035)
Rhizobium hainanense CCBAU 57015 (FMAC01000030)
Rhizobium tropici CIAT 899 (CP004015)
Rhizobium multihospitium HAMB 2975 (1052913)
Cupriavidus taiwanensis LMG 19424(NR 074823.1)
0.10
recA
Bradyrhizobium sp. SEMIA 6069 (FJ391162.1)
XBQ5 (OQ266919)
Bradyrhizobium sp. rhw4 (KF532839.1)
Bradyrhizobium sp. CCBAU 33039 (KC356517.1)
Bradyrhizobium sp. CCBAU 41003 (KC356524.1)
Bradyrhizobium elkanii Cte-503 (MH982259.1)
Bradyrhizobium elkanii USDA76 (AY591568.1)
Bradyrhizobium icense LMTR 13 (JX943615.1)
Bradyrhizobium paxllaeri LMTR 14 (KF896166.1)
Bradyrhizobium betae PL7HG1(FJ970378.1)
Bradyrhizobium diazoefficiens NUJ N43 (MK246834.1)
Bradyrhizobium diazoefficiens USDA 122 (CP013127.1)
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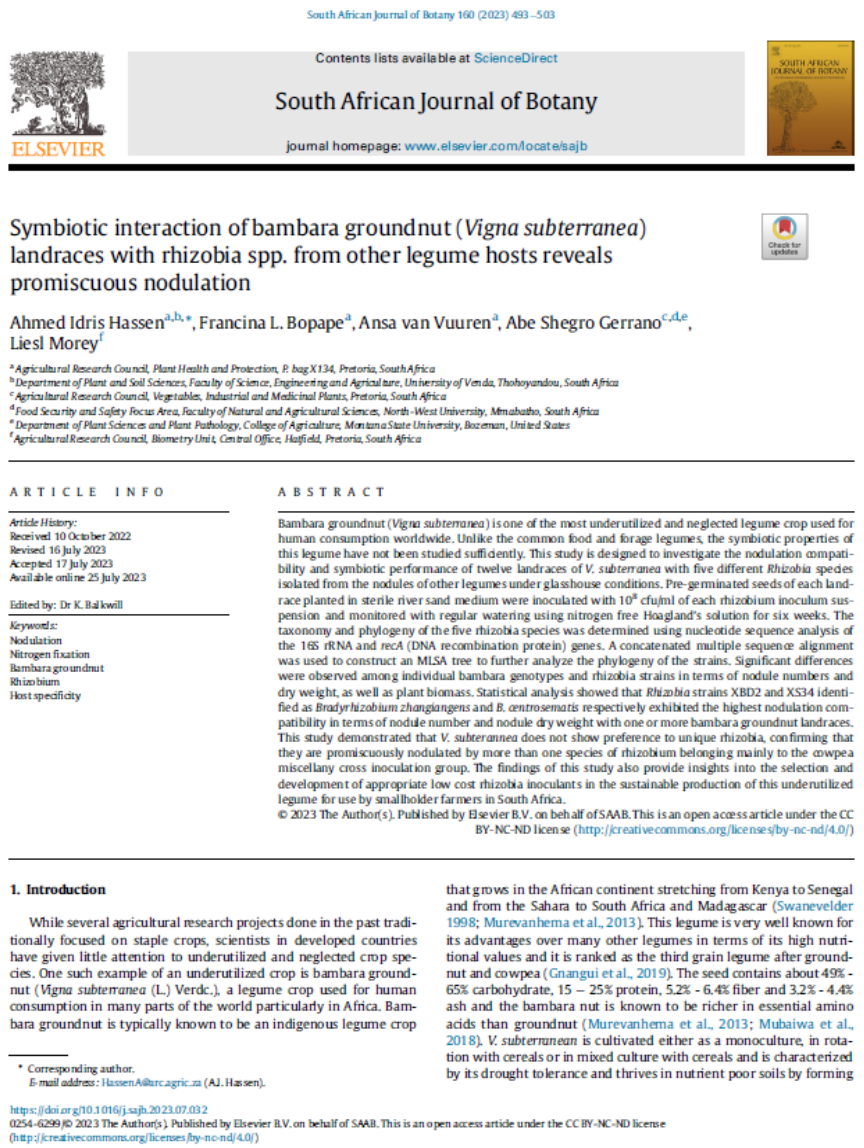
Bradyrhizobium japonicum USDA122 (AY591562.1)
WB1 (OQ266918)
Bradyrhizobium iriomotense ARR602 (LM994254.1)
Bradyrhizobium kavangense 14-3 (KM378399.1)
Bradyrhizobium frederickii CNPSo 3426 (MK682710.1)
Bradyrhizobium nanningense CCBAU 53390 (KC509274.1
Bradyrhizobium centrosematis A9 (KC247145.1)
Bradyrhizobium guangxiense CCBAU 53363 (KC509279.1)
Bradyrhizobium canariense CCBAU 51257 (GU433529.1)
Bradyrhizobium zhanjiangense CCBAU 51787 (KX683408.1)
Phyllobacterium rubiacearum ATCC 43591(AJ294
Rhizobium laguerreae FB206 (JN558681.2)
Bradyrhizobium pachyrhizi VAm13-2 (LC107306.1)
Rhizobium hainanense CCBAU 57015 (HM047132.1)
Rhizobium tropici strain Hm7 (KU175244.1)
Rhizobium tropici strain RHM14 (JQ085274.1)
XBD2 (ON673126)
Rhizobium tropici strain BR 322 (MN272181.1)
Rhizobium tropici strain BR 850 (JF318198.1)
UD5 (ON673113)
XS34 (ON673111)
Rhodobacter sphaeroides (X72705)
0.050
MLSA
Bradyrhizobium icense LMTR 13
Bradyrhizobium paxllaeri LMTR 14
Bradyrhizobium elkanii USDA 76
Bradyrhizobium elkanii Cte-503
Bradyrhizobium sp.SEMIA 6069
Bradyrhizobium pachyrhizi VAm13-2
Bradyrhizobium sp. CCBAU 33039
XBQ5 (SARCC-401)
Bradyrhizobium iriomotense ARR602
Bradyrhizobium kavangense 14-3
Bradyrhizobium zhanjiangense CCBAU 51787
Bradyrhizobium canariense CCBAU 51257
Bradyrhizobium diazoefficiens USDA122
Bradyrhizobium japonicum SEMIA 5080
Bradyrhizobium centrosematis A9
Bradyrhizobium betae PL7HG1
Bradyrhizobium frederickii CNPSo3426
Bradyrhizobium nanningense CCBAU 53390
Bradyrhizobium guangxiense CCBAU 53363
XS34 (SARCC-578)
Cupriavidus taiwanensis LMG 19424
Rhizobium laguerreae FB206
Rhizobium tropici Hm7
UD5 (SARCC-715)
Rhizobium hainanense strain CCBAU57015
XBD2 (SARCC-388)
WB1(SARCC-304)
Rhizobium tropici USDA 9030
2.00

Concluding Remark

- **Limited information on the symbiotic interaction of *V. subterranea* to nodulate with other rhizobia within or outside of the crop inoculation group**
- **Three of the legumes (*Desmodium uncinatum, Cymopsis tetragonoloba* and *Arachis hypogeae*) belong to the same CIR with *Vigna subterranea***
- **Assumption that bambara groundnut could be nodulated by rhizobia isolated from legumes within its CIR**



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Symbiotic interaction of bambara groundnut (*Vigna subterranea*) landraces with rhizobia spp. from other legume hosts reveals promiscuous nodulation

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ABSTRACT

Bambara groundnut (*Vigna subterranea*) is one of the most underutilized and neglected legume crop used for human consumption worldwide. Unlike the common food and forage legumes, the symbiotic properties of this legume have not been studied sufficiently. This study is designed to investigate the nodulation compatibility and symbiotic performance of twelve landraces of *V. subterranea* with five different *Rhizobia* species isolated from the nodules of other legumes under glasshouse conditions. Pre-germinated seeds of each landrace planted in sterile river sand medium were inoculated with 10^8 cfu/ml of each rhizobium inoculum suspension and monitored with regular watering using nitrogen free Hoagland's solution for six weeks. The taxonomy and phylogeny of the five rhizobia species was determined using nucleotide sequence analysis of the 16S rRNA and *recA* (DNA recombination protein) genes. A concatenated multiple sequence alignment was used to construct an MLSA tree to further analyze the phylogeny of the strains. Significant differences were observed among individual bambara genotypes and rhizobia strains in terms of nodule numbers and dry weight, as well as plant biomass. Statistical analysis showed that *Rhizobia* strains XBD2 and XS34 identified as *Bradyrhizobium zhangiangens* and *B. centrosematis* respectively exhibited the highest nodulation compatibility in terms of nodule number and nodule dry weight with one or more bambara groundnut landraces. This study demonstrated that *V. subterannea* does not show preference to unique rhizobia, confirming that they are promiscuously nodulated by more than one species of rhizobium belonging mainly to the cowpea miscellany cross inoculation group. The findings of this study also provide insights into the selection and development of appropriate low cost rhizobia inoculants in the sustainable production of this underutilized legume for use by smallholder farmers in South Africa.

1. Introduction

While several agricultural research projects done in the past traditionally focused on staple crops, scientists in developed countries have given little attention to underutilized and neglected crop species. One such example of an underutilized crop is bambara groundnut (*Vigna subterranea* (L.) Verdc.), a legume crop used for human consumption in many parts of the world particularly in Africa. Bambara groundnut is typically known to be an indigenous legume crop that grows in the African continent stretching from Kenya to Senegal and from the Sahara to South Africa and Madagascar (Swanevelder 1998; Murevanhema et al., 2013). This legume is very well known for its advantages over many other legumes in terms of its high nutritional values and it is ranked as the third grain legume after groundnut and cowpea (Gnangui et al., 2019). The seed contains about 49% - 65% carbohydrate, 15 – 25% protein, 5.2% - 6.4% fiber and 3.2% - 4.4% ash and the bambara nut is known to be richer in essential amino acids than groundnut (Murevanhema et al., 2013; Mubaiwa et al., 2018). *V. subterranean* is cultivated either as a monoculture, in rotation with cereals or in mixed culture with cereals and is characterized by its drought tolerance and thrives in nutrient poor soils by forming

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Concluding Remark……

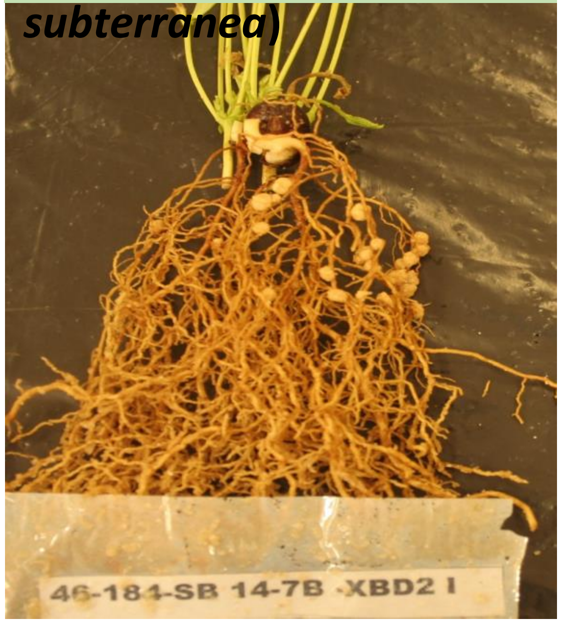
- **Three, out of the five rhizobia strains induced nodulation in more than one landrace**
- **Despite the variation in symbiotic performance, rhizobia strains from *Arachis hypogeae* (XS34), *Cyamopsis tetragonoloba* (XBD2), *Desmodium uncinatum* (XBQ5) formed pink, effective nodules on *V. subterranea***
- **The least nodulation compatibility (or not at all) with bambara groundnut was exhibited by rhizobia strains from *Phaseolus vulgaris* (UD5) and *Glycine max* (WB1)**
- **Strains XBD2, XBQ5 and XS34 have very distinct *phylogenetic placement* on the NJ tree**
- **Indication that bambara groundnut promiscously nodulates and form effective symbiosis with phylogenetically diverse group of rhizobia**
- **XBD2 temporarily classified as *Bradyrhizobium zhanjiangense* XBD2 (= SARCC-388)**

Concluding Remark……

Cowpea (*V. unguiculata*)

Hassen et al. 2014; 2023

Bambara (*V. subterranea*)



- **High potential as a commercial inoculant for under-utilized legumes in the cowpea miscellany CIG**
- **Prospects for use in sustainable and climate smart agriculture with a boost for the Bioeconomy**
- **The finding of this study is highly relevant to smallholder farmers who produce under-utilized legumes such as bambara groundnut on poor soils with no application of inoculants.**

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Thank You