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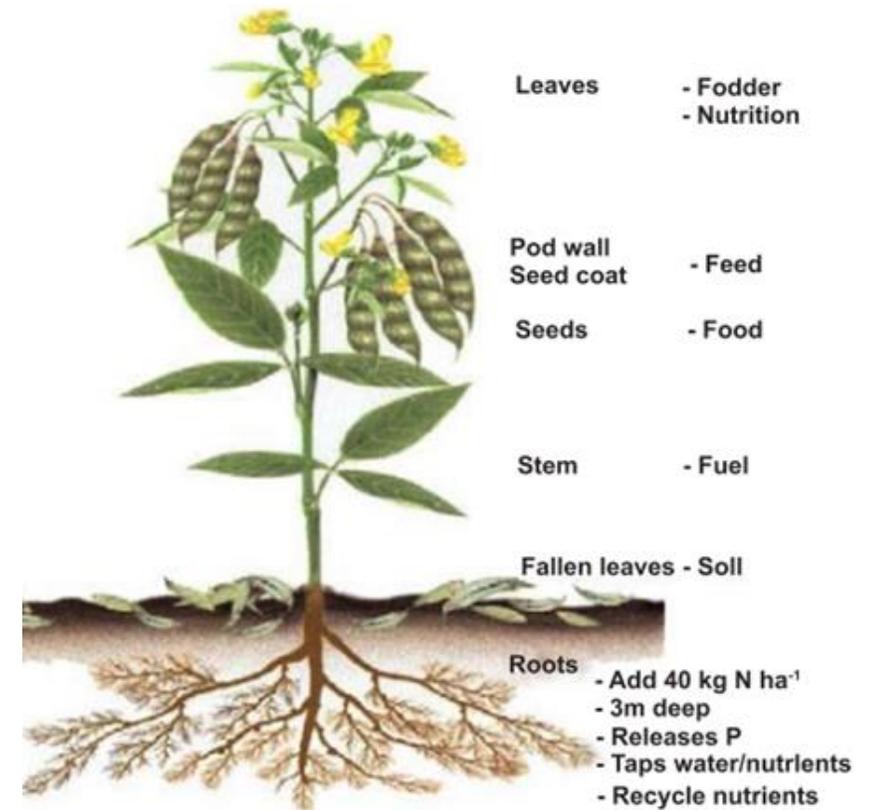
## Evaluation of Root Nodulation in Pigeonpea using indigenous Soil Rhizobia and Symbiotic Performance with Pigeonpea and other Common Tropical Legumes

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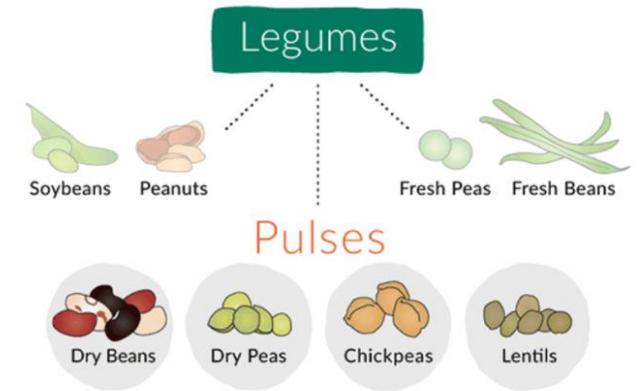
# Presentation outline

1. Introduction
2. Problem statement and justification
3. Aim and Specific Objectives
4. Materials and Methods
5. Results and Discussion
6. Conclusions and recommendations
7. Acknowledgements



# Introduction

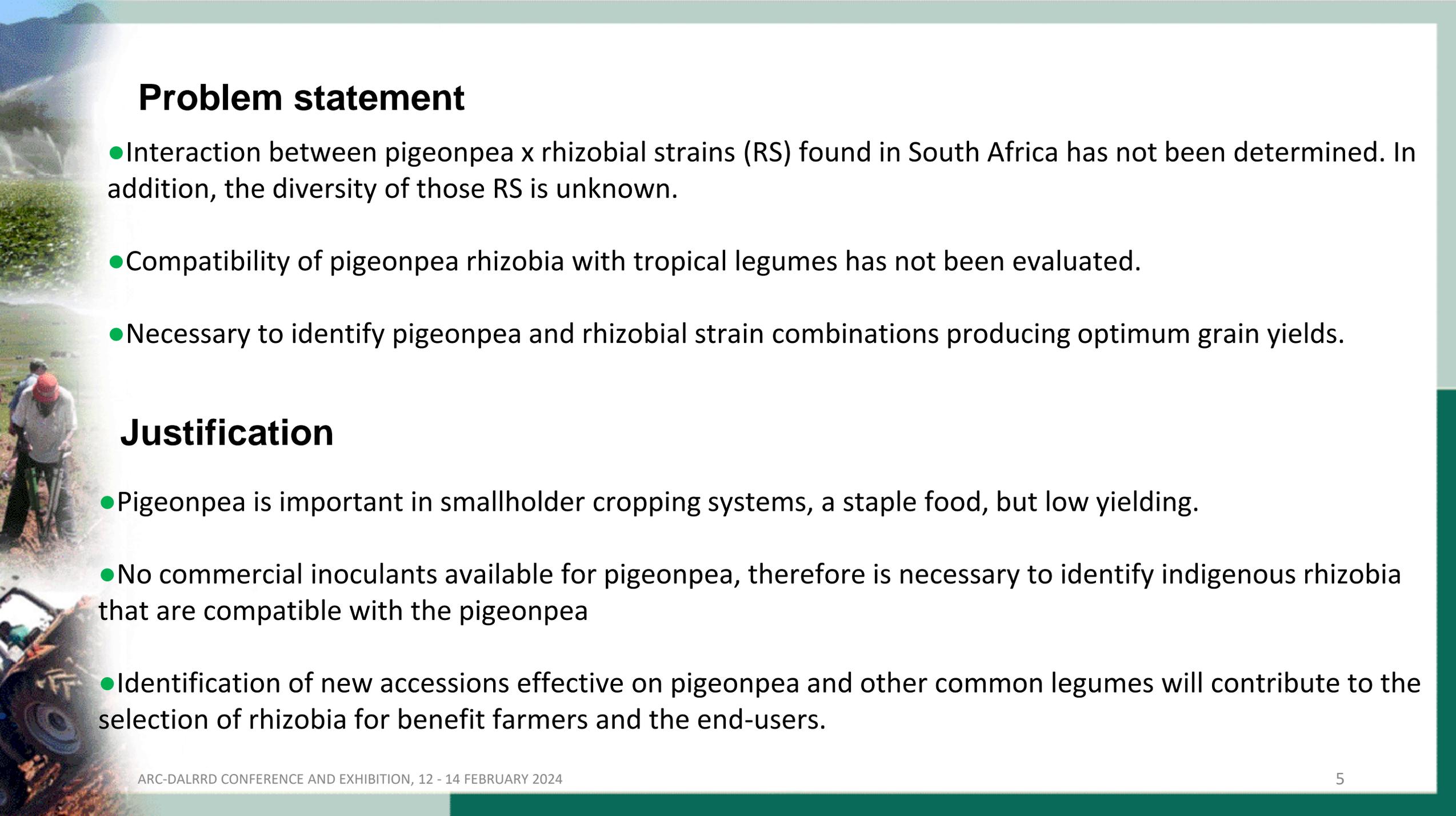
- Pigeonpea (*Cajanus cajan*) is an important pulse
- Origin India, 3500 years ago (Patel et al., 2010).
- A low cost crop, grown in Africa countries
- Production in Africa dominated in the Eastern and S.A (Ayenan et al., 2017)
- Minor production in South Africa
- Productivity is low (0.4 t/ha), in smallholder farmers
- Due to environmental and genetic factors (Kumar et al., 2009), highly tolerant to drought (Emefiene et al., 2013).
- The grains are highly rich in nutrients



# Introduction

- Multiple diverse uses as animal feeds, human food.
- Pigeonpea grains reduce poverty as a protein source food
- Pigeonpea meets its nitrogen requirement by fixing atmospheric nitrogen ( $N_2$ ) in symbiotic association with the root nodule bacteria.
- The determination of host legume x rhizobial combinations that produce optimum growth and crop yield is important for food security.
- Pigeonpea improves soil health, fertility and quality (Mapfumo et al., 1999).
- Host range symbiotic performance of rhizobia on common tropical legumes is important in order to assess cross compatibility





## Problem statement

- Interaction between pigeonpea x rhizobial strains (RS) found in South Africa has not been determined. In addition, the diversity of those RS is unknown.
- Compatibility of pigeonpea rhizobia with tropical legumes has not been evaluated.
- Necessary to identify pigeonpea and rhizobial strain combinations producing optimum grain yields.

## Justification

- Pigeonpea is important in smallholder cropping systems, a staple food, but low yielding.
- No commercial inoculants available for pigeonpea, therefore is necessary to identify indigenous rhizobia that are compatible with the pigeonpea
- Identification of new accessions effective on pigeonpea and other common legumes will contribute to the selection of rhizobia for benefit farmers and the end-users.

## Aim and Specific Objectives

### Aim:

To evaluate the symbiotic effectiveness of indigenous rhizobia strains on pigeonpea and tropical legumes to improve growth and grain yield

### The objectives were to:

- (i) Collect rhizobial strains that are associated with pigeonpea root nodulation and improved growth
- (ii) Morphological and molecular characterization of rhizobia isolated from pigeonpea
- (iii) Determine the symbiotic effectiveness of indigenous rhizobia with common tropical legume species.



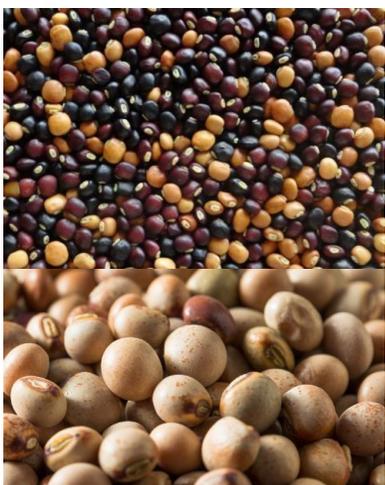
## Materials and methods

- Forty soil samples (200.0g each) were collected in South Africa
- Soil with no history of pigeonpea cultivation



## Materials and methods

- Used to isolate rhizobia by the soil-trap inoculation method using five pigeonpea cultivars
- Medium duration pigeonpea genotypes (3 local and 2 improved)
- Pigeonpea plants raised in nitrogen-free medium in the greenhouse
- After 6 weeks of growth, the plants were harvested
- Isolates from root nodules evaluated morphologically on YMA-CR media





## Materials and methods

- DNA extracted from isolates and 16S rRNA PCR was performed for genus identity determination.
- And *recA* PCR conducted in order identify the isolates to species level
- Rhizobia isolates derived from pigeonpea were assessed for their host range symbiotic performance with the common tropical legumes
- That included soybean (*Glycine max*), Bambara groundnut (*Vigna subterranea* ), Tepary bean (*Phaseolus acutifolius*) and pigeonpea (*Cajanus cajan*).
- The datasets for N<sub>2</sub> fixation parameters analysed with ANOVA and GGE biplot

## Results and discussion



Fig. 1 The uninoculated (control) pigeonpea plants (red circles) showing chlorotic leaves



Fig. 2 No nodules on uninoculated plants, nodules on roots of inoculated pigeonpea

# Results and discussion

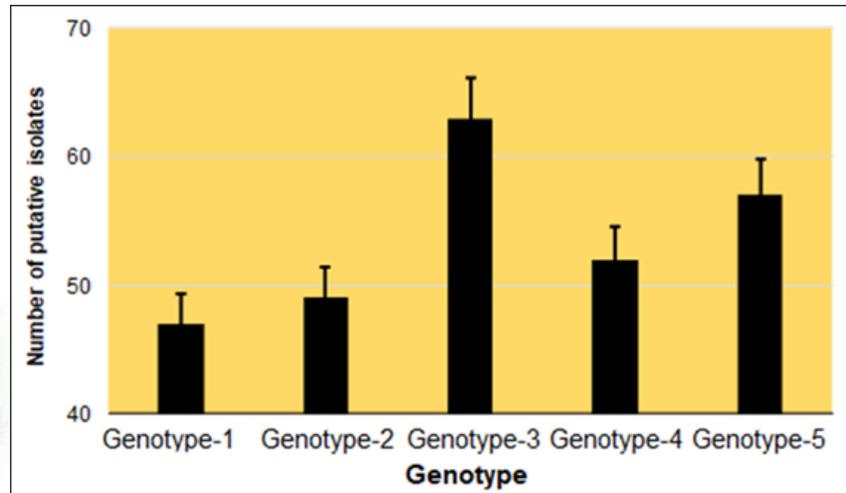


Fig. 3 Approximate distribution of putative rhizobial isolates derived from the root nodules of five distinct pigeonpea genotypes.

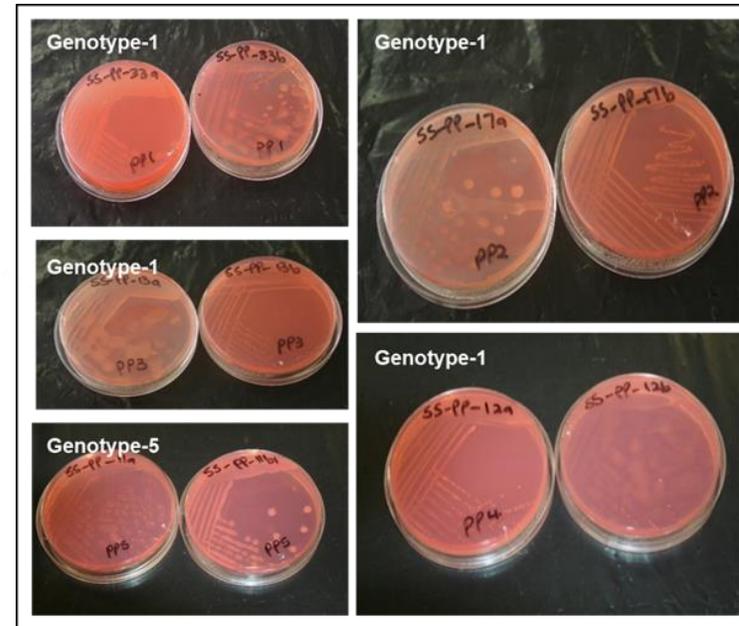


Fig. 4 Variations in the morphology of pure cultures of rhizobial isolates (growing on YMA-CR).

# Results and discussion

Table 1 The mean squares for root nodulation traits among five pigeonpea genotypes inoculated with different soil samples

Source	df	NN	NFW	NDW	RFW	RDW	SFW	SDW
Rep (R)	2	52.99	6.88**	5.05**	0.19	0.31	0.02	0.00
Genotype (G)	4	105.53**	3.46**	8.66**	29.23**	0.43**	0.15**	0.01**
Isolate (I)	40	556.16**	4.91**	1.34**	2.95**	0.37**	0.04**	0.01**
Genotype x Isolate	160	40.11**	0.61*	0.86*	1.21**	0.04*	0.02*	0.00**

NN = nodule number; NFW = nodule fresh weight; NDW = nodule dry weight; RDW = root dry weight; RFW = root fresh weight; SFW = shoot fresh weight; SDW = shoot dry weight. \*, \*\* significance at 5.0 % and 1.0% probability levels respectively.

# Results and discussion

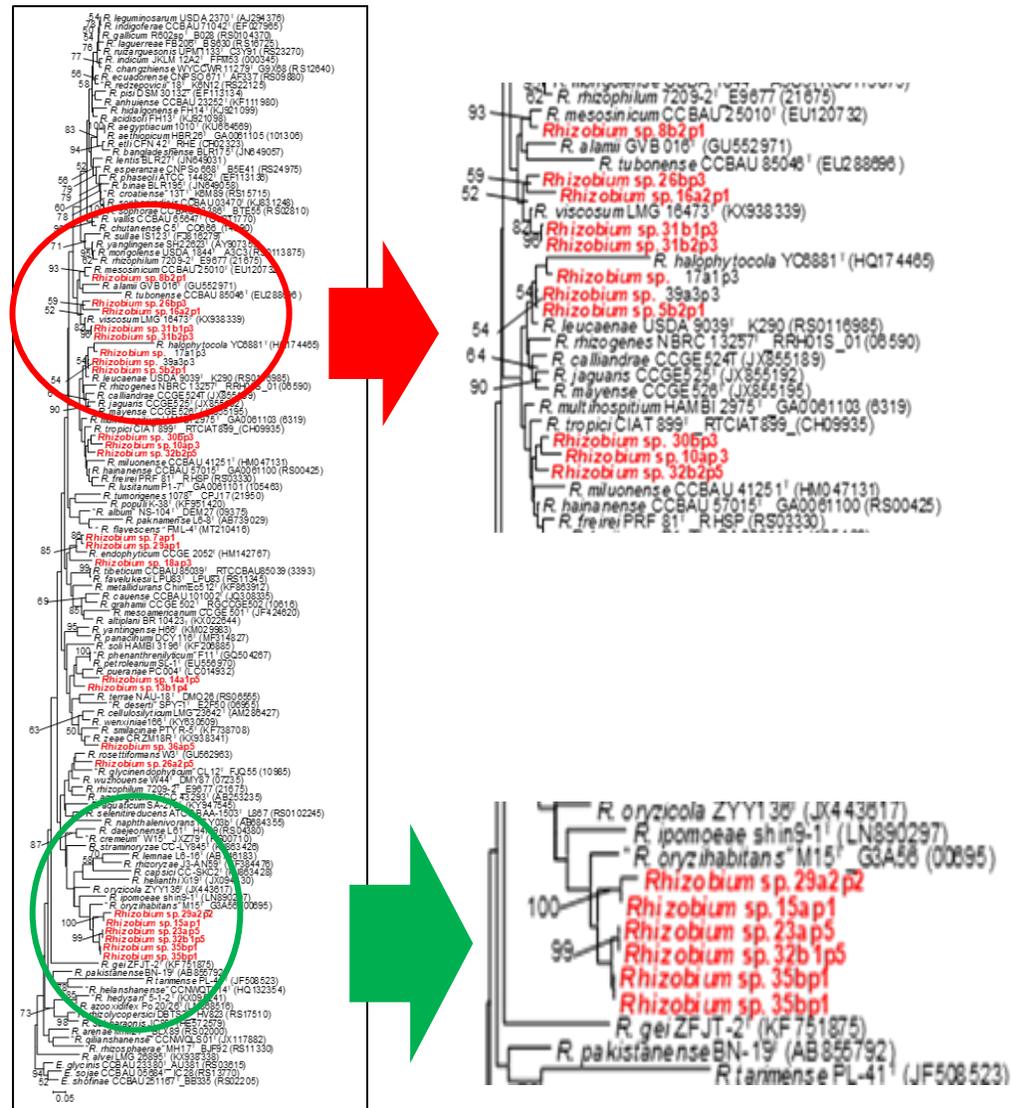
Entry #	Isolate	Genus	Accession number	Sequence size
1	10ap3	<i>Rhizobium</i>	OK376715	1393 bp
2	30bp3	<i>Rhizobium</i>	OK384644	1391 bp
3	37ap4	<i>Rhizobium</i>	OP013029	931 bp
4	29a1p2	<i>Rhizobium</i>	OK384642	771 bp
5	23ap5	<i>Rhizobium</i>	OK384609	1397 bp
6	18ap3	<i>Rhizobium</i>	OK384614	921 bp
7	17ap1	<i>Rhizobium</i>	OK377268	1386 bp
8	29ap1	<i>Rhizobium</i>	OK384643	805 bp
9	39a3p3	<i>Rhizobium</i>	OP013028	794 bp
10	17a1p3	<i>Rhizobium</i>	OK377065	920 bp
11	5b2p1	<i>Rhizobium</i>	OK376606	1470 bp
12	35ap5	<i>Rhizobium</i>	OK384675	1394 bp
13	35ap3	<i>Rhizobium</i>	OK384671	854 bp
14	35bp1	<i>Rhizobium</i>	OK384700	1389 bp
15	32b2p5	<i>Rhizobium</i>	OK384673	1392 bp
16	32b1p5	<i>Rhizobium</i>	OK384666	1395 bp
17	26a2p5	<i>Rhizobium</i>	OK384615	1393 bp
18	15ap1	<i>Rhizobium</i>	OK377037	1393 bp
19	31b1p5	<i>Rhizobium</i>	OK384652	1396 bp
20	13b1p4	<i>Rhizobium</i>	OP013027	807 bp
21	14a1p5	<i>Rhizobium</i>	OP013030	814 bp
22	8b2p1	<i>Rhizobium</i>	OK376646	1395 bp
23	31b1p3	<i>Rhizobium</i>	OK384650	1397 bp
24	31b2p3	<i>Rhizobium</i>	OK384662	1398 bp
25	16a2p1	<i>Rhizobium</i>	OK377114	878 bp
26	26bp3	<i>Rhizobium</i>	OK384638	1387 bp
27	36ap5	<i>Rhizobium</i>	OK384704	853 bp
28	22ap5	<i>Rhizobium</i>	OK384604	655 bp
29	5ap4	<i>Bradyrhizobium</i>	OP010913	808 bp
30	8a2p3	<i>Bradyrhizobium</i>	OK392643	1391 bp
31	27b2p5	<i>Bradyrhizobium</i>	OK393634	1390 bp
32	11a2p3	<i>Bradyrhizobium</i>	OP010914	921 bp
33	7a2p3	<i>Bradyrhizobium</i>	OK392633	827 bp
34	15bp3	<i>Bradyrhizobium</i>	OP010916	947 bp
35	19bp5	<i>Bradyrhizobium</i>	OK393635	1401 bp
36	15bp5	<i>Bradyrhizobium</i>	OP010918	926 bp
37	33ap4	<i>Bradyrhizobium</i>	OK393623	854 bp
28	11b2p5	<i>Bradyrhizobium</i>	OP010915	810 bp
39	13bp3	<i>Bradyrhizobium</i>	OK392642	502 bp
40	19a1p3	<i>Bradyrhizobium</i>	OP010917	745 bp
41	30a2p3	<i>Paraburkholderia</i>	OK393667	1434 bp
42	31bp4	<i>Phyllobacterium</i>		798 bp
43	32a1p2	<i>Rhizobium</i>		967 bp
44	29a2p2	<i>Rhizobium</i>		435 bp
45	15ap3	<i>Rhizobium</i>		888 bp
46	16a2p3	<i>Bradyrhizobium</i>		827 bp
47	6bp3	<i>Bradyrhizobium</i>		806 bp
48	18bp5	<i>Rhizobium</i>		551 bp
49	38a1p5	<i>Rhizobium</i>		1046 bp
50	34a2p5	<i>Rhizobium</i>		563 bp
51	27ap1	<i>Rhizobium</i>		966 bp
52	32bp2	<i>Rhizobium</i>		688 bp
53	31b3p3	<i>Rhizobium</i>		711 bp
54	30b2p2	<i>Rhizobium</i>		705 bp
55	14bp5	<i>Rhizobium</i>		679 bp
56	22ap1	<i>Rhizobium</i>		926 bp

Table 2 16S rRNA pigeonpea rhizobial sequences deposited into the national gene bank



# Results and discussion

Fig. 6 A *recA* maximum-likelihood phylogeny of *Rhizobium* isolates associated with pigeonpea. Bootstrap support for the groupings above 50% is indicated. The scale bar corresponds to the number of nucleotide changes per site.



## Results and discussion

- A marked variability in the responses of tropical legume species to inoculations with rhizobial strains
- Indicating that compatibility with the rhizobial inoculations varied between legumes



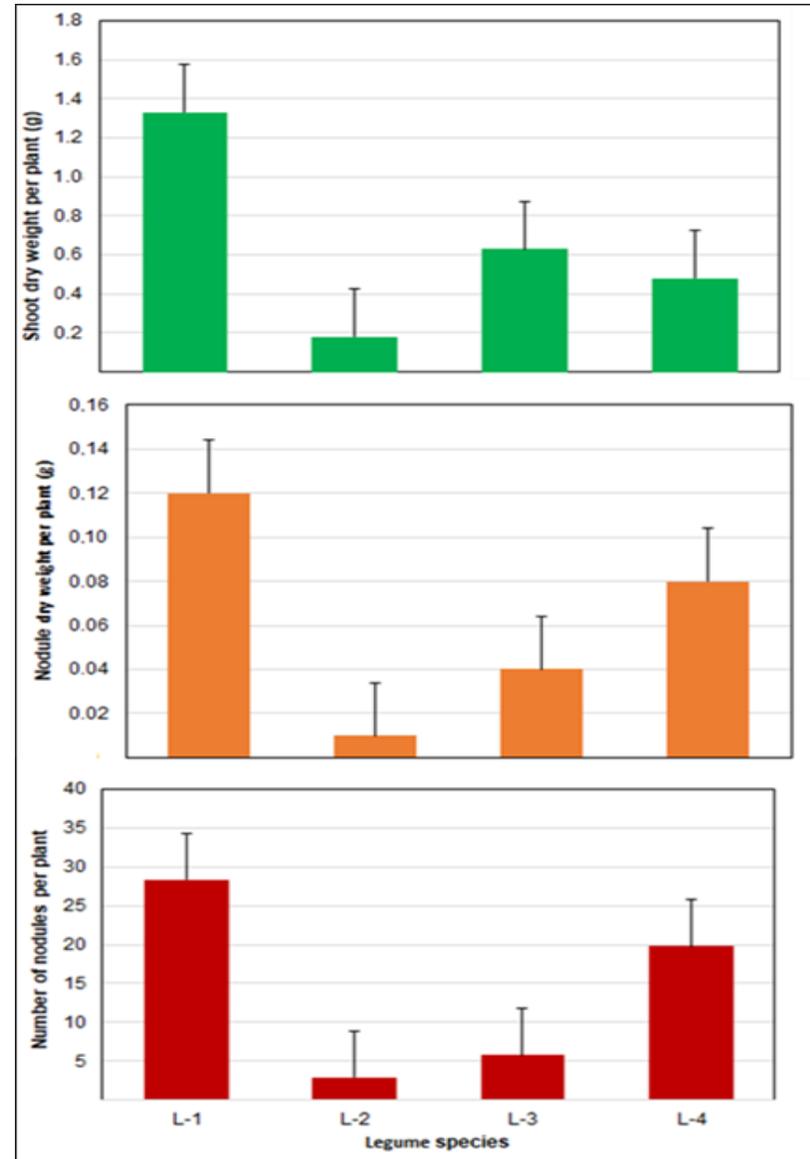
Fig. 7 Variation in leaf color between (a) pigeonpea and (b) soybean plants inoculated with specific rhizobial strains.



Fig. 8 Contrast in response to inoculation with rhizobial strains in (a) tepary bean with chlorotic leaves and (b) Bambara groundnut with dark green leaves.

# Results and discussion

Fig. 9 The mean number of nodules (bottom), nodule dry weight (middle) and shoot dry weight per plant among four tropical legumes that were inoculated separately with specific rhizobial strains. (L-1 = Bambara groundnut; L-2 = tepary bean; L-3 = soybean; L-4 = pigeonpea).



## Results and discussion

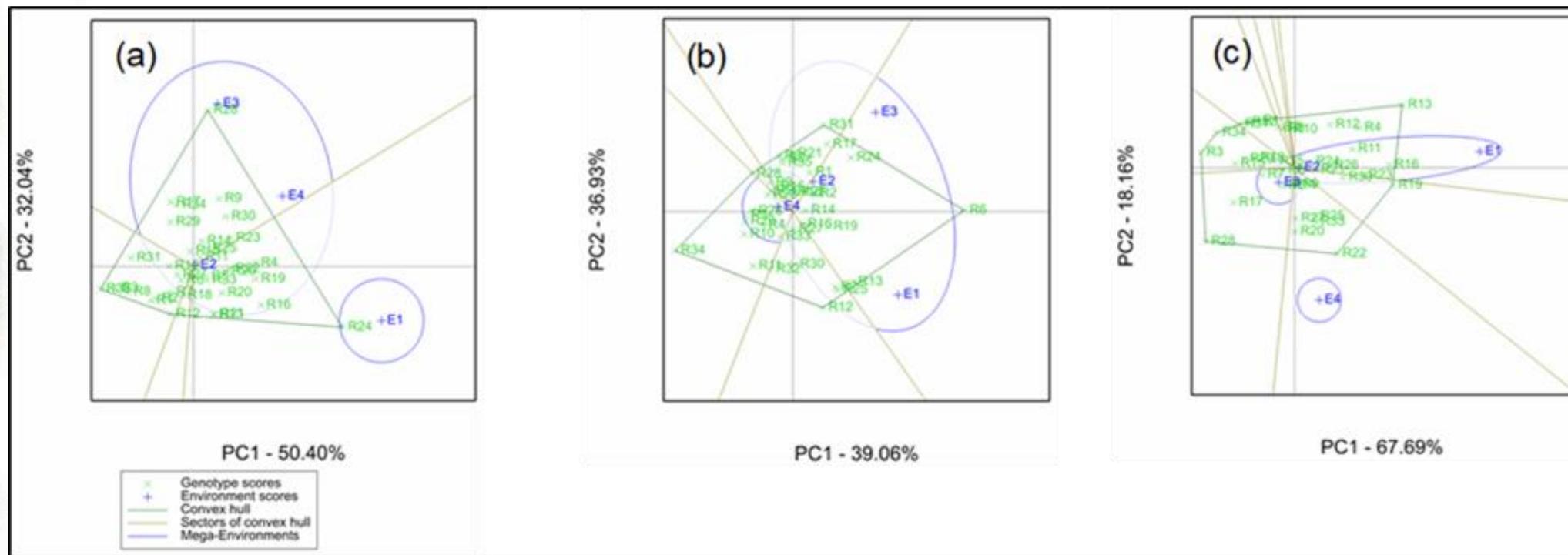


Fig. 10 GGE biplot analysis of 'which-won-where' among rhizobial strains (coded as rhizobial scores) that were used for inoculating each of four tropical legume species (coded as environment scores) for (a) nodule dry weight (b) root dry weight and (c) shoot dry weight; E1 = Bambara groundnut; E2 = tepary bean; E3 = soybean; E4 = pigeonpea.

# Conclusions

- South African soil contain diverse rhizobial isolates effective on pigeonpea
- There was potential to select for an optimum pigeonpea x rhizobial isolate combinations for N fixation and improve legume grain yield.
- Two rhizobial strains (Rhizobium sp. 26a2-PP5 and 36a-PP5) performed best in inducing NDW on soybean and Bambara groundnut
- The GGE biplot analysis also revealed that both pigeonpea and Bambara groundnut provided the most ideal symbiotic activity for NDW

## Recommendations

- The study demonstrated promiscuous nodulation of pigeonpea and cross-compatibility of pigeonpea rhizobia with other tropical legumes.
- Two Rhizobium performed best with Bambara groundnut and soybean, which will improve yields on these crops
- Further testing and validation of the symbiotic activities of the rhizobial strains identified in this study in field trials in multiple agro-ecological locations is recommended.
- This could lead to possible expansion of pigeonpea production area in South Africa and hence an improvement in the quantity of pigeonpea productivity and production in the country as well as food security
- Essential to exploit the identified rhizobial strains (accessions) from this study in developing commercial bio-inoculants for pigeonpea and other common tropical legumes
- It will also be desirable to identify new rhizobia (bio-inoculants) for improving tepary bean productivity in South Africa

# Research Outputs

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## Short Communication

### Symbiotic efficiency of pigeonpea (*Cajanus cajan*) with different sources of nitrogen

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GENOME SEQUENCES



### Draft Genome Sequence of *Rhizobium tropici* SARCC-755, a Free-Living Rhizobium That Nodulated and Promoted Growth in Pigeonpea [*Cajanus cajan* (L.) Millsp.]

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## Article

### Symbiotic Performance and Characterization of Pigeonpea (*Cajanus cajan* L. Millsp.) Rhizobia Occurring in South African Soils

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## Correction

### Correction: Bopape et al. The Genome of a Pigeonpea Compatible Rhizobial Strain '10ap3' Appears to Lack Common Nodulation Genes. *Genes* 2023, 14, 1084

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