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

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Abstract: Pigeonpea (*Cajanus cajan* (L.) Millsp.) is an important grain legume, which, like several other legumes, depends on the process of biological nitrogen fixation for its nitrogen (N_2) requirement by forming a symbiotic association with rhizobia. Compared to other tropical legumes, however, the productivity of pigeonpea in South Africa is low, despite the extensive interests in developing it for wider markets. To assist this process, the objectives of the current study were to (i) characterize putative indigenous rhizobial strains that were previously derived from local soils with no previous history of legume cultivation and (ii) confirm their nodulation abilities on a local landrace and a genetically improved (exotic) genotype of pigeonpea. DNA-based analyses using the 16S rRNA and *recA* genes showed that the strains predominantly represented *Rhizobium* and *Bradyrhizobium*, although we also recovered *Phyllobacterium* and *Paraburkholderia*. These rhizobia nodulated both the local landrace and the improved pigeonpea genotype that were included for comparative purposes. In many cases, rhizobia performed similarly on the two genotypes, although the locally sourced landrace mostly performed better in terms of nodulation and plant biomass. While the current study generated vital information regarding the diversity of indigenous rhizobia associating with pigeonpea, further screening (including field inoculation trials) would be necessary to identify possible elite nitrogen fixing rhizobial strains for development as inoculants to enhance South African pigeonpea production.

Keywords: grain legume; nodulation; plant growth promotion; alphaproteobacteria; betaproteobacteria

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1. Introduction

Biological nitrogen fixation is one of the most important processes necessary for plant growth and yield improvements. This property is associated with two of the six subfamilies (i.e., Papilionoideae and Caesalpinioideae) of the Leguminosae/Fabaceae [1]. Specifically, these legumes make use of symbiotic nitrogen fixation (SNF), which relies on the association with a group of soil bacteria capable of inducing the formation of novel plant structures called nodules on either the stem or roots of the host, within which the fixation process happens [2]. Bacteria with the ability to induce such nitrogen-fixing nodules are referred to as rhizobia [3].

Like most other legumes, pigeonpea (*Cajanus cajan*) also derives its nitrogen mainly through the process of SNF [4,5]. Pigeonpea is widely cultivated for human consumption as a source of protein and food grain particularly in low-income communities. It serves as a valuable forage and green manure crop, and its cultivation contributes to soil fertility

through SNF [6–9]. Due to its consumption by both humans and livestock, pigeonpea can be traded in both informal and formal markets, thereby generating household income [10]. However, the productivity of pigeonpea is generally low, particularly in smallholder cropping systems [11–13]. For example, in Tanzania an average yield of only 0.4 t/ha was reported for the growing season of 2002 to 2003 [12].

In terms of its agronomic and economic value, pigeonpea is a low-input crop with no chemical fertilizer requirements, as nitrogen is supplied through the natural symbiosis with rhizobia [14]. The legume is generally compatible with rhizobia that form part of the cowpea miscellany group, which commonly also nodulate legumes such as cowpea (*Vigna unguiculata*), siratro (*Macropitilium atropurpureum*), lima bean (*Phaseolus lunatus*), and peanut (*Arachis hypogaea*) [15–18]. This broad host-range group is indigenous to African soils and represent slow-growing rhizobia from the genus *Bradyrhizobium* [19]. Various studies from Africa have also shown that the interactions between these rhizobia and their legume hosts are promiscuous, especially in terms of their symbiosis with peanut, cowpea, and cowpea relatives [19–22]. However, despite the general lack of host-specificity in the cowpea miscellany group, all its members are not equally able to nodulate different legumes. For example, in one of the first studies to characterize this trait, some symbionts of groundnut and *Vigna* species could also nodulate pigeonpea, while others could not [18].

Pigeonpea is among the most drought-tolerant legumes and regarded as having great potential for cultivation in the water-stressed and semi-arid regions of Africa [23]. Although its origins are still debatable, the species is thought to have been introduced from India to East Africa, from where it was moved to other parts of Africa [23]. This includes South Africa, where perennial varieties of pigeonpea are predominantly grown as single plants in home gardens in the Kwazulu-Natal, Limpopo and Mpumalanga provinces [24]. However, in recognition of its potential for cultivation in the drought prone regions of South Africa, local stakeholders are using various initiatives to increase awareness about the benefits of pigeonpea cultivation and to promote it as a commercial crop in South Africa [25].

An important factor in pigeonpea cultivation is the availability of compatible rhizobia to allow efficient SNF. It rarely requires rhizobial inoculants due to the legume's inherent promiscuity making it compatible with resident soil rhizobia [11,23], and thus, commercial inoculants are not available for this crop. In other words, the limited instances of pigeonpea cultivation in South Africa are achieved without the application of inoculum, as the legume associates with compatible rhizobia already present in the soil [26,27]. There is a dearth of reliable information regarding the identity and diversity of the rhizobial partners of pigeonpea, especially from South Africa, where the crop is cultivated on a non-commercial scale. Rhizobia are polyphyletic and consist of species which belong to 18 genera of the alphaproteobacteria (alpha-rhizobia) and beta-proteobacteria (beta-rhizobia) [28,29]. However, previous studies elsewhere indicated that pigeonpea was associated with rhizobia from several genera including *Bradyrhizobium* spp. [30], *Mesorhizobium* [31], *Rhizobium* spp. [32,33] *Sinorhizobium*/Ensifer [34,35]. Although the *Paraburkholderia* genus consists of mostly plant-beneficial bacteria, including strains that are capable of SNF [36], some of the strains from this genus were associated mainly with mimosoid legumes [37,38]. However, little is known regarding the host range of rhizobia native to South African soils, nor whether they would sustain SNF with pigeonpea. This is particularly relevant given that not all cowpea miscellany group rhizobia are equally capable of nodulating pigeonpea. It would thus be important to determine whether South African soils contain rhizobia suitable for pigeonpea nodulation and nitrogen fixation. Such knowledge would be invaluable for improving production in the current growing areas as well as for the expansion of production into new areas in the country.

Therefore, the goal of this study was to characterize individual indigenous rhizobial strains that were previously derived from local soils with no previous history of legume cultivation and confirm their nodulation abilities on a locally sourced landrace and a genetically improved genotype of pigeonpea. This was achieved by using putative

pigeonpea-nodulating rhizobia that were isolated using trapping experiments with South African soils and diverse genotypes of the legume in a previous study [26].

2. Materials and Methods

2.1. Pigeonpea Genotypes and Rhizobial Strains

Two piegonepea genotypes were used in the study to confirm their nodulation abilities with each of the putative rhizobial strains. The genotypes consisted of a locally sourced landrace (PP1-3018) and an improved genotype (PP5-3021) (Table 1). A set of 39 randomly selected putative rhizobial strains was used in the study for inoculating the seed of the pigeonpea genotypes during planting as described below (Table 2). All the putative rhizobial strains were obtained originally from soil samples (that were collected from diverse locations across South Africa) and used for inoculating and trapping experiments involving five distinct pigeonpea genotypes in a glasshouse [26].

Table 1. Pigeonpea germplasm used in the study.

Genotype	Seed Colour	Status (Source)
PP1-3018	Grey	Unimproved landrace (locally sourced)
PP5-3021	White/Cream	Improved germplasm, (International Crops Research Institute for the Semi-Arid Tropic)

2.2. Glasshouse Trial Establishment

The seeds of each pigeonpea genotype were pretreated with 70% ethanol for 30 s, and surface sterilized with 3.5% sodium hypochlorite for 3 min. After rinsing 5 times with sterile distilled water, the seeds were imbibed in sterile distilled water for 3–4 h at room temperature. The seeds were then germinated overnight at 28 °C on water agar medium containing 20 g/L bacteriological agar (Merck, Midrand, South Africa). Three seeds of each genotype were planted separately in a 2.0 L Leonard jar containing sterilized sand and nitrogen-free Hoagland's plant growth solution [39]. The soil was saturated with sterile distilled water, followed by planting the seeds and inoculating each genotype separately with each of the rhizobial strains [26]. The Leonard jars were then placed in a temperature-controlled glasshouse with a day length set at 14 h and temperature set at 28 °C, and night length set at 10h and temperature set at 20 °C.

2.3. Experimental Design, Measurements and Statistical Analysis

A randomized complete block design with three replications was used for the study. After six weeks of growth, prior to harvesting, the color of leaves and root nodules were assessed for evidence of nitrogen fixation. Plants with yellow, chlorotic leaves were expected to either lack nodules or have ineffective nodules with white interiors suggesting absence of nitrogen fixation [40]. In contrast, the plants with dark green leaves as well as nodules with pink to red interiors indicated successful nitrogen fixation. The latter is indicative of the presence of leghemoglobin, a protein produced by the host essential to the function of the oxygen-sensitive nitrogenase [41]. Upon harvesting the plants, four nitrogen fixation variables, namely, number of root nodules (NN), nodule fresh weight (NFW), root fresh weight (RFW), and shoot fresh weight (SFW), were measured for each plant after which the nodule dry weight (NDW), root dry weight (RDW), and shoot dry weight (SDW) were determined following oven-drying to a constant weight at 70 °C. The quantitative data sets for each of the seven nitrogen fixation variables for each pigeonpea genotype were analyzed using the standard analysis of variance procedure followed by mean separation at the 5.0% probability level of the least significance difference (LSD) test in the Statistical Analysis Software (SAS version 9.1, Cary, NC, USA) package [42].

Table 2. Rhizobial strains used in the study.

Rhizobial Strain		Rhizobial Species
Code	Designated Name	
R1	8b2p1	*Rhizobium* sp.
R2	8a2p3	*Bradyrhizobium* sp.
R3	32b2p5	*Rhizobium* sp.
R4	32b1p5	*Rhizobium* sp.
R5	6bp3	*Bradyrhizobium* sp.
R6	7a2p3	*Bradyrhizobium* sp.
R7	30bp3	*Rhizobium* sp.
R8	30a2p3	*Paraburkholderia* sp.
R9	11a2p3	*Bradyrhizobium* sp.
R10	10ap3	*Rhizobium* sp.
R11	39a3p3	*Rhizobium* sp.
R12	16a2p1	*Rhizobium* sp.
R13	15ap1	*Rhizobium* sp.
R14	18ap3	*Rhizobium* sp.
R15	35ap3	*Rhizobium* sp.
R16	35bp1	*Rhizobium* sp.
R17	37ap4	*Rhizobium* sp.
R18	5b2p1	*Rhizobium* sp.
R19	27b2p5	*Bradyrhizobium* sp.
R20	31b1p5	*Rhizobium* sp.
R21	31b2p3	*Rhizobium* sp.
R22	31b1p3	*Rhizobium* sp.
R23	31ap4	*Phyllobacteriumsp.*
R24	38a1p5	*Rhizobium* sp.
R25	33ap4	*Bradyrhizobium* sp.
R26	17ap1	*Rhizobium* sp.
R27	17a1p3	*Rhizobium* sp.
R28	14a1p5	*Rhizobium* sp.
R29	13b1p4	*Rhizobium* sp.
R30	13bp3	*Bradyrhizobium* sp.
R31	23ap5	Rhizobium sp.
R32	19bp5	*Bradyrhizobium* sp.
R33	36ap5	*Rhizobium* sp.
R34	34a2p5	*Rhizobium* sp.
R35	22ap1	*Rhizobium* sp.
R36	29ap1	*Rhizobium* sp.
R37	29a1p2	*Rhizobium* sp.
R38	29a2p2	*Rhizobium* sp.
R39	26bp3	*Rhizobium* sp.
R40	Control	water

2.4. DNA-Based Identification of Rhizobial Strains

Representatives of the rhizobial strains that were previously isolated from pigeonpea were identified using DNA sequences for both the 16S ribosomal RNA (rRNA) subunit gene and the *recA* house keeping gene [43–45]. To achieve this, the respective bacteria were used to inoculate tubes containing 5 mL of sterile tryptone yeast extract broth (TYB), which consisted of 5 g/L tryptone (Oxoid, Midrand, South Africa), 3 g/L yeast extract (Biolab, Midrand, South Africa), and 15 g/L agar (Merck, Midrand, South Africa). The inoculated tubes were incubated for 24 to 48 h at 28 °C and 150 rpm on a rotary shaker. Bacterial cells were then harvested by centrifugation and subjected to DNA extraction using the WIZARD genomic DNA purification kit (Promega, Madison, WI, USA).

The extracted DNA was used to PCR amplify the relevant regions. For 16S rRNA, the primers 27F (5′ AGA GTT TGA TCC TGG CTC AG 3′) and 1485R (5′ TAC CTT GTT ACG ACT TCA CCC CA 3′) were employed [46]. Each 50 µL-reaction 5 ng/µL DNA, 1.5 mM $MgCl_2$, 800 µM dNTPs, 0.5 µM of each primer and 0.025 U/µL SuperTherm Taq polymerase

(Promega, Madison, WI, USA) together with its reaction buffer. PCR amplification was carried out in an Eppendorf Master Cycler Gradient apparatus (Applied Biosystems, San Francisco, CA, USA). The cycling conditions entailed and initial denaturation at 94 °C was for 4 min, followed by 35 cycles of denaturation at 94 °C for 1 min, annealing at 60 °C for 30 s, and extension at 72 °C for 1 min, after which a final extension step was performed at 72 °C for 1 min.

For the *recA* PCR, four different sets of primers were used. The primers recA63F ('5 ATC GAG CGG TCG TTC GGC AAG GG 3′) and recA504R ('5 TTG CGC AGC GCC TGG CTC AT 3′) were used to amplify a portion of *recA* in strains representing *Bradyrhizobium*, *Paraburkholderia*, and *Phyllobacterium*, as well as some strains of *Rhizobium* [46]. For Rhizobium strains, we also used primers recA6F ('5 CGK CTS GTA GAG GAY AAA TCG GTG GA 3′) and recA555R ('5-CGR ATC TGG TTG ATG AAG ATC ACC AT 3′). In both these cases, the reaction mixtures were constituted the same as for the 16S rRNA PCRs (except that the relevant *recA* primers were used) and optimum cycling conditions were applied [46]. For those strains in which the initial two sets of *recA* primers did not work, we used a third set of primers, TSrecAf5 ('5 CAC TGC MYT GCG TAT YGT CGA AGG 3′) and TsrecAr3 ('5 GAT CTT CAT SCG GAT CTG GTT GATG 3′) [46]. For these reactions, we employed FastStart High-Fidelity *Taq* and its $MgCl_2$ and buffer (Roche Diagnostics, Midrand, South Africa) and optimum cycling conditions were applied.

All PCR products were purified with Exonuclease 1 and FASTAP alkaline phosphatase (Thermo Scientific, Johannesburg, South Africa). The PCR products were sequenced in both directions (using the original PCR primers) with an ABI PRISM BigDye Terminator v3.1 Cycle Sequencing Kit on an ABI3100 Automated Capillary DNA sequencer (Applied Biosystems, San Francisco, CA, USA). All raw sequence files were inspected and edited using Chromas Lite version 2.0 (Technelysium, Brisbane, Australia) and BioEdit version 5.0.9 [47]. The 16S rRNA sequence for each strain was compared to all publicly available sequences in the GenBank database of the National Centre for Biotechnology Information using the BLASTN software [48]. A separate dataset was constructed for representatives for each of the genera recovered in this study. The type strain information necessary to construct the datasets was obtained from a list of prokaryotic names with standing in nomenclature [49] and included multiple-sequence alignment files together with the pigeonpea rhizobial sequences generated in this study. All sequences generated during this study were submitted to National Center for Biotechnology Information using the Genbank submission portal National Institute of Health and were assigned the following accession numbers: 16S rRNA OK376606-OK384775, OK392633-OK393635, OP010913-OP010918, and OP013027-OP013030.

All 16S rRNA datasets were aligned with the online server of MAFFT (multiple alignment using fast Fourier transformation [50]), taking secondary structure into account by using the Q-INS-I strategy [51]. The *recA* datasets were aligned manually in BioEdit, based upon the inferred amino acid sequences. Sequences which were too short for meaningful comparison (for type strains as well as pigeonpea strains) were excluded from the alignments. To determine the appropriate evolutionary models for the alignments, jModelTest v2.1.7 was used [52,53]. For those datasets that did not use the general time reversible (GTR) model, maximum-likelihood analyses were performed in PhyML v3.1 while the analysis of those datasets using this model was performed with PhyML v.3.0 on the Montpellier bioinformatics platform [54]. Branch support was evaluated using bootstrap analysis [55] performed using 1000 pseudoreplicates.

3. Results

3.1. Glasshouse Nodulation Test Experiment

Bacterial strains from the current study were tested for nodulation on an indigenous pigeonpea landrace (PP1-3018) and an improved genotype (PP5-3021). Effective nodulation was evident from the plants' healthy appearance and green color, while those in which nodulation was ineffective or lacking were small, with yellow to light green leaves (Figure 1).

Inspection of roots also revealed that the healthy plants had numerous nodules with pink interiors, while those of the less healthy plants lacked nodules or harboured ineffective, small nodules, lacking leghemoglobin. The uninoculated control plants were also stunted with yellow leaves, and their roots did not have nodules. In all cases, inoculation of the two genotypes and subsequent DNA sequence analysis confirmed that the inocula used in the various treatments were indeed responsible for the observed phenotypes.

Figure 1. Pigeonpea plants of the (**a**) local landrace genotype PP1-3018 and (**b**) the exotic improved genotype PP5-3021. Genotype PP1-3018 appears a much healthier with a dark green color, while some of the plants of genotype PP5-3021 appear less healthy with light green/yellow leaves; (**c**) most of the plants with yellow leaves, as well as the uninoculated controls, lacked nodules (**left**) while the healthy-looking plants had roots bearing numerous indeterminate nodules (**right**).

Of the set of 39 strains that were used in the study, 32 nodulated the indigenous landrace effectively, while 34 effectively nodulated the improved genotype. Of the 32 which nodulated the indigenous genotype, 1–32 nodules/plant were produced on average, depending on the bacterial inoculum used. For those nodulating the improved genotype, this ranged from 1–27 nodules/plant, depending on the bacterial inoculum used. The highest NN was induced by the rhizobial strain 10ap3 (identified as *Rhizobium* sp 10) (Figure 2). In certain cases, strains capable of inducing most nodules and yielding the highest biomass on the indigenous genotype, were also able to do so on the improved genotype. Significant examples are strains 10ap3 (identified as *Rhizobium* sp 10) and 16a2p1 (identified as *Rhizobium* sp 12) (Figure 2).

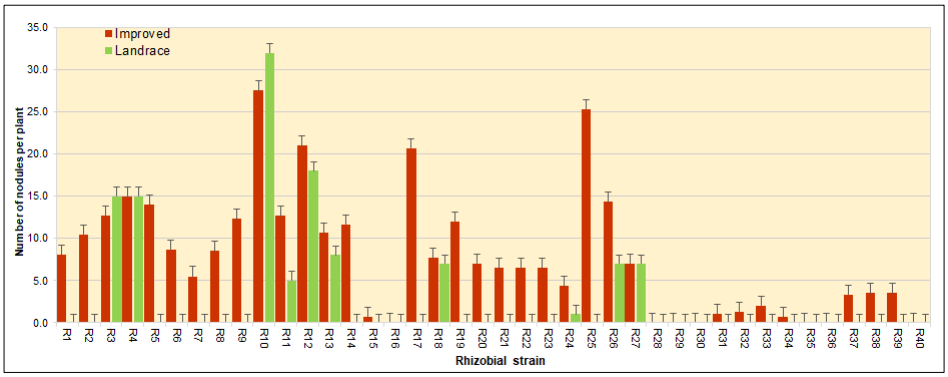

Figure 2. The effects of inoculation with rhizobial strains on the number of nodules per plant of the landrace and improved pigeonpea genotypes. The identity of each rhizobial strain (R1–R40) is described in Table 2 and the results of the statistical analysis are reported in Tables S1 and S2 (depending on the genotype).

The results also showed that the *Bradyrhizobium* strain 13bp3 (coded R30) produced NDW with the landrace genotype, but not with the improved genotype (Figure 3). In contrast, two *Rhizobium* sp (22ap1 and 29ap1) produced no detectable NDW with both pigeonpea genotypes (Figure 3). The SDW was similar between the landrace and the improved genotype with some of the rhizobial strains, for instance *Phyllobacterium* sp 31ap4 (code R23) and *Paraburkholderia* sp 30a2p3 (coded R8) (Figure 4). Two *Bradyrhizobium* strains (7a2p3 and 33ap4) and one *Rhizobium* strain (39a3p3) induced significantly high RDW in the improved genotype while inoculation with the *Rhizobium* sp 30bp3 resulted in a significantly high RDW in the landrace genotype (Figure 5). The respective fresh weights (of the nodules, shoots, and roots) showed some variable responses among the two pigeonpea genotypes but were considered less reliable indicators of nitrogen fixation (Supplementary Tables S1 and S2).

3.2. DNA-Based Identification of Rhizobial Strains

To identify the bacteria at species level, we utilized the phylogenies inferred from the 16S rRNA (diagram not shown) and *recA* alignments that were generated for the *Rhizobium* (Figure 6) and *Bradyrhizobium* (Figure 7). For this purpose, phylogenetic distances among known species were compared to those among the strains from this study. This approach was used in combination with the species delineation method which assumes that members of a species would consistently group together in different gene trees [21,34]. The strains that belong to the same genus or species mostly formed the same grouping either on the 16S rRNA or the *recA* phylogeny. For instance, in the *Rhizobium* genus, the *Rhizobium* sp 5b2p1, 10ap3, and 30bp3 formed the same cluster with *R. lusitanum*, *R. multihospitium* and *R. tropici* in the *recA* tree (Figure 6). In addition, the *Bradyrhizobium* strain 33ap4 is a close relative of *B. ferriligni* and *B. elkanii* (Figure 7). From the phylogenetic standpoint, these approaches revealed that all the strains isolated in the current study are likely new.

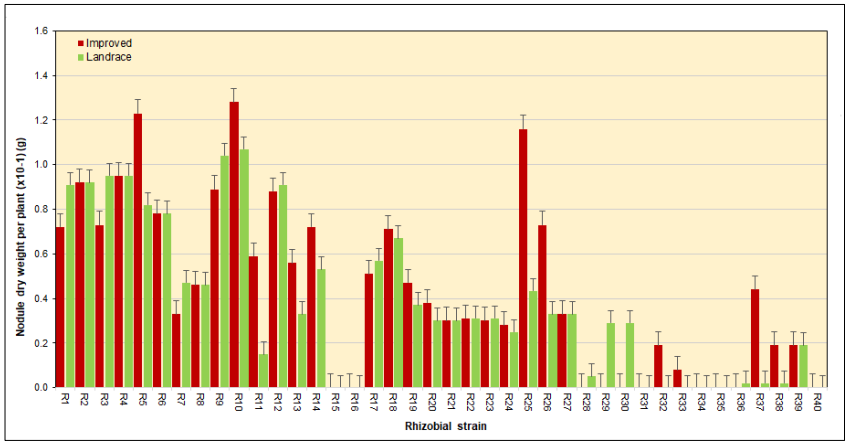

Figure 3. The effects of inoculation with rhizobial strains on the nodule dry weight per plant of the landrace and improved pigeonpea genotypes. The identity of each rhizobial strain (R1–R40) is described in Table 2 and the results of the statistical analysis are reported in Tables S1 and S2 (depending on the genotype).

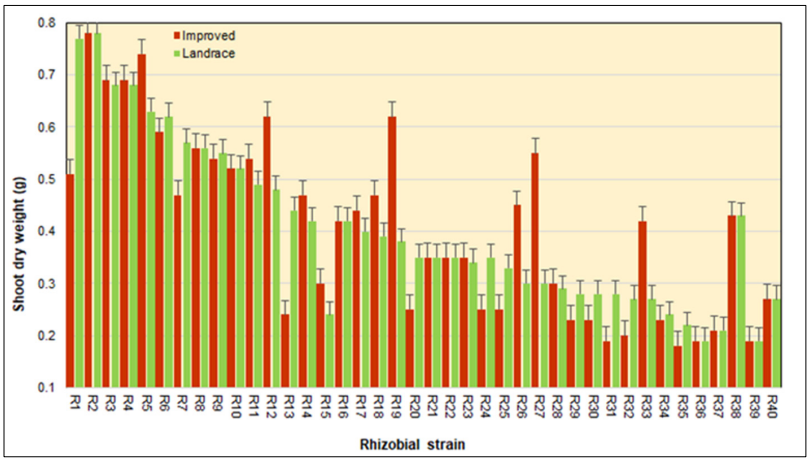

Figure 4. The effects of inoculation with rhizobial strains on the shoot dry weight per plant of the landrace and improved pigeonpea genotypes. The identity of each rhizobial strain (R1–R40) is described in Table 2 and the results of the statistical analysis are reported in Tables S1 and S2 (depending on the genotype).

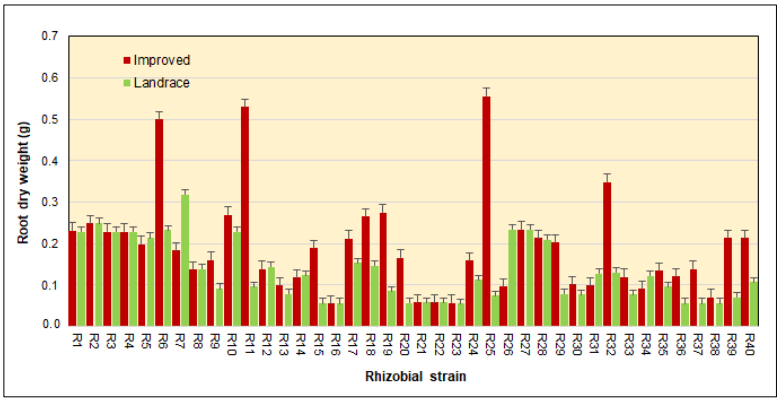

Figure 5. The effects of inoculation with rhizobial strains on the root dry weight per plant of the landrace and improved pigeonpea genotypes. The identity of each rhizobial strain (R1–R40) is described in Table 2 and the results of the statistical analysis are reported in Tables S1 and S2 (depending on the genotype).

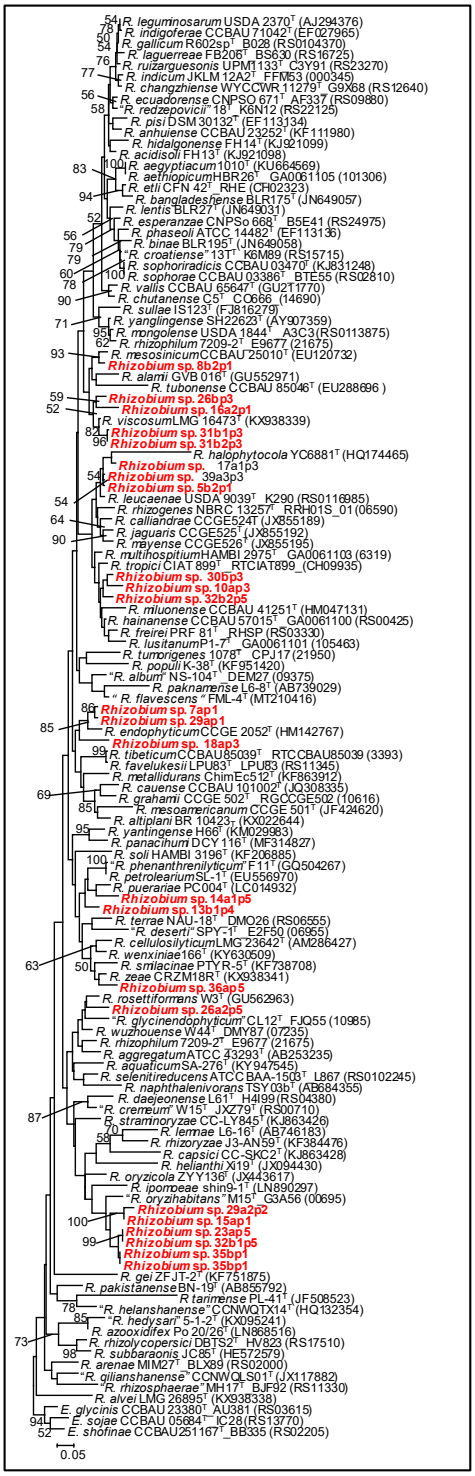

Figure 6. A *recA* maximum-likelihood phylogeny of *Rhizobium* strains associated with pigeonpea. The strains in red originated from pigeonpea nodules. Bootstrap support for the groupings above 50% are indicated. Information regarding the type strain is provided while the GenBank accession number or locus tag for each species is indicated in brackets. Species names which appear in inverted commas (' . . . ') are combinations which have not yet been validly published. The scale bar corresponds to the number of nucleotide changes per site. The model used is GTR + I + G with invariable proportion site value 0.4310 and gamma value 0.8190.

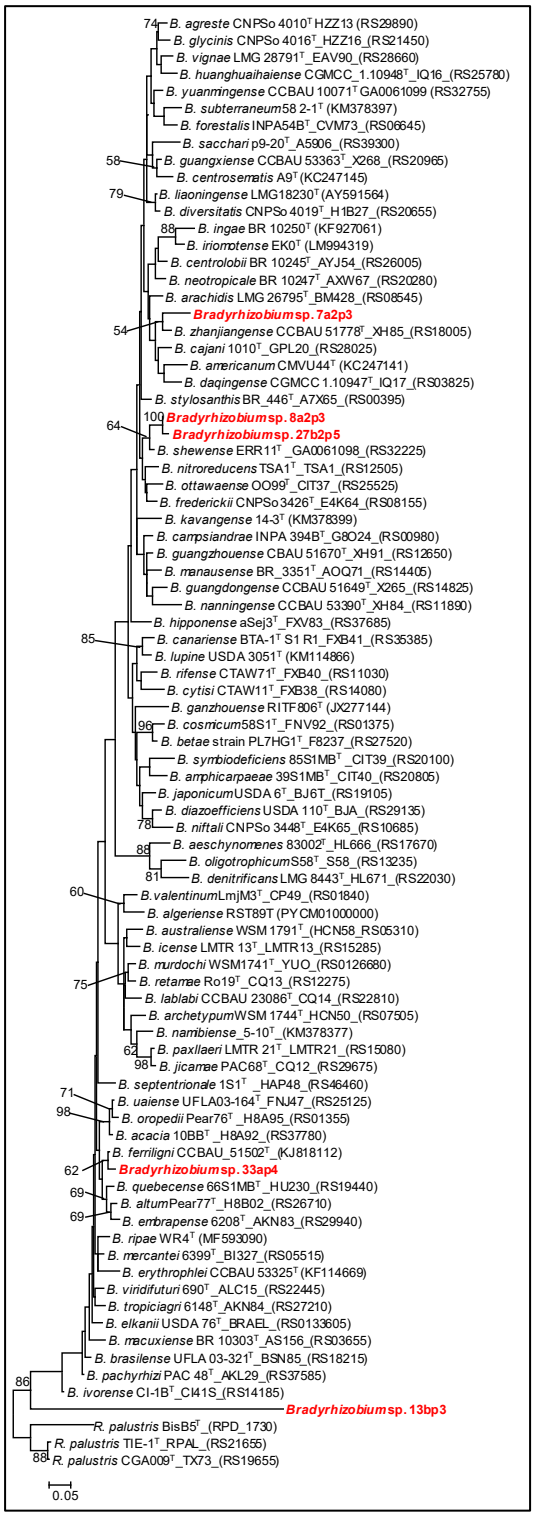

Figure 7. A *recA* maximum-likelihood phylogeny of *Bradyrhizobium* strains associated with pigeonpea. The strains in red originated from pigeonpea nodules. Bootstrap Bootstrap support for the groupings above 50% are indicated. Information regarding the type strain is provided while the GenBank accession number or locus tag for each species is indicated in brackets. Species names which appear in inverted commas (' . . . ') are combinations which have not yet been validly published. The scale bar corresponds to the number of nucleotide changes per site. The model used is GTR+ I+ G with invariable proportion site value 0.4986 and gamma value 0.6930.

4. Discussion

This study characterized the rhizobial strains which were originally sampled from diverse locations (virgin or grasslands transects) that had no history of pigeonpea cultivation across South Africa. In these regions, some studies on edible legumes such as cowpea, dry bean, and soybean were conduted previously, but little is known about their symbiotic efficiency with pigeonpea [24]. We thus aimed to characterize pigeonpea-nodulating bacteria from soils that were previously collected from different regions of South Africa, and then evaluate their ability to nodulate and promote the growth of pigeonpea using an indigenous and an improved genotype (Figure 3). The results of the nodulation test indicated that some rhizobial strains that were evaluated (for instance, *Bradyrhizobium* strain 13bp3) could nodulate with the indigenous genotype (Figure 3) but could not nodulate with the improved genotype, thus suggesting that some soils had strains with low nodulation and nitrogen fixation efficiencies as compared to soils from other locations such as those with strains 23ap5, 34a2p5, and 22ap1. Similarly, some rhizobial strains (35bp1, 14a1p5 and 22ap1) failed to nodulate the improved genotype suggesting that there was host genotype x rhizobial strain incompatibility between some strains and the improved pigeonpea genotype. Nevertheless, there were nodulation compatibilities with similar rhizobial strains, such as 32b2p5, 10ap3, and 16a2p1, in both genotypes.

Our results revealed that diverse bacteria from both the α-proteobacteria and β-proteobacteria may occupy the root nodules of pigeonpea. We further assembled confirmed rhizobia, belonging to distinct genera, namely, *Rhizobium, Bradyrhizobium,* and *Phyllobacterium*, and one strain from the genus *Paraburkholderia* in the β-proteobacteria. In addition to the rhizobia, other non-rhizobial bacteria were also isolated and partially identified with 16S rRNA. These mostly represented *Bacillus*, *Pseudomonas, Burkholderia,* and *Paenibacillus*, which have no known history of nodulation [56]. However, these genera are known to contain endophytes and plant growth promoting rhizobacteria (PGPR) [57]. Another study identified endophytes in nodules of groundnut improving plant growth [58]. In addition, PGPR, *Burkholderia* sp. Nafp2/4-1b (SARCC-3049) could improve the growth of maize, while coinoculation of *Burkholderia* and rhizobia on *Medicago sativa* increased the number of nodules and plant biomass of *Medicago sativa* [59]. Future research should thus seek to investigate their potential for promoting the growth of pigeonpea, as previous work has shown that they can benefit legume growth in various ways.

Rhizobium and *Bradyrhizobium* appear to be common symbionts of pigeonpea (Figure 2) across the sample areas in South Africa. This was consistent with the observation that pigeonpea-nodulating rhizobial strains native to African soils belong to the broader *B. japonicum* and *B. elkanii* groups [60]. In addition, slow-growing *Bradyrhizobium* sp of the cowpea miscellany group and fast-growing *Rhizobium* sp nodulated pigeonpea [61]. Similarly, other studies identified *Bradyrhizobim* species as a commonly nodulating pigeonpea on the coast of West Africa in Côte d' Ivoire, while *Rhizobium* species commonly nodulated pigeonpea in semi-arid regions of India [33,62].

Our study also showed for the first time that members of *Phyllobacterium* and *Paraburkholderia* can nodulate pigeonpea (Figure 3). In previous studies, endophytic strains of the genus *Paraburkholderia* that exhibited antimicrobial potential against *Fusarium udum* were isolated from the leaves, roots, and stems of pigeonpea [63]. Nonetheless, some strains from this genus that could fix N were associated mainly with mimosoid legumes [37,38]. Similarly, there are no reports of symbiotic strains of *Phyllobacterium* that were isolated from pigeonpea. Therefore, this legume is thus compatible with diverse symbionts, and some of these less commonly encountered bacteria might have potential in agriculture. Moreover, the availability of diverse inoculants would contribute to the expansion of pigeonpea to new areas. The indigenous genotype of pigeonpea performed better than the improved genotype. Our findings correlate with those from other studies, which also found that pigeonpea can grow well in natural environments without artificial inoculation [64]. The indigenous pigeonpea genotype interacted more effectively with indigenous soil rhizobia in comparison to the imported genotype. Therefore, indigenous rhizobia and unimproved

pigeonpea genotypes may be are better suited to one another when grown in these environments. However, field trails will be necessary before proposing these strains for commercial inoculum production. They could also be tested on more genotypes, with the emphasis on climate change, e.g., by testing at higher temperatures and under semi-arid conditions.

In conclusion, this study revealed that pigeonpea is compatible with diverse groups of rhizobia occurring in South Africa which include both α- and β-proteobacteria as their symbiotic partners. Furthermore, *Rhizobium* and *Bradyrhizobium* from South African soils are the common symbionts of pigeonpea. In addition, the indigenous pigeonpea genotypes appear to be more compatible with the indigenous strains than the improved genotypes. For future studies, several strains from this study can be used for co-inoculating pigeonpea seed to determine if a significant improvement in the crop productivity will be achieved.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/agriculture13010030/s1, Table S1: The nodule fresh weight of the improved genotype and landrace of pigeonpea; Table S2: The shoot fresh weight of the improved genotype and landrace of pigeonpea.

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