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Genetic diversity and symbiotic efficiency of rhizobial strains isolated from nodules of peanut (*Arachis hypogaea* L.) in Senegal

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1. Introduction

Cultivated peanut (*Arachis hypogaea* L.) was domesticated in South America. The value of the crop was quickly perceived and it has been successfully adapted to many other tropical and subtropical zones worldwide (Hammons, 1994). Today peanut is cultivated on approximately 25 million hectares from which 45 million tons are harvested annually (FAOSTAT, 2014) making peanut one of the most cultivated grain legumes and a crucial source of protein, oil and revenue. In Senegal peanut cultivation started in the 1850s. Since the 1960s, peanut is cultivated as an industrial export-oriented crop, representing up to 80% of Senegalese exports and providing the majority of cash income for rural populations (Sene et al., 2010; Noba et al., 2014).

Like many other legumes, *A. hypogaea* is able to establish a mutualistic symbiotic association with soil bacteria collectively known as rhizobia. Rhizobia form symbioses with legumes and fix atmospheric nitrogen, converting it into a form that can be assimilated by plants (Mylona et al., 1995). Grain legumes generally assimilate around 80% of their nitrogen from the air, but peanut only acquires by this way about 55% of its total nitrogen requirement (Hardarson, 1993). In addition, fixation efficiency is highly variable between peanut genotypes, rhizobia strains, and environmental conditions (Hardy et al., 1968; Stalker, 1991; Stalker et al., 1994; Mokgehle et al., 2014). This variability is certainly linked to the intrinsic characteristics of each partner and to the capacity of host plants to recognize and retain the best (Frederickson, 2013; Ibáñez et al., 2016, 2015), but could also reflect a complex evolutionary history of hosts and symbionts including horizontal transfer of symbiotic genes that created genotypes adapted to particular geographic zones or hosts (Laguerre et al., 2001; Muñoz et al., 2011).

Although peanut has long been adapted to the Senegalese agro-systems, little is known about the native symbiotic components that allow for biological nitrogen fixation (Sene et al., 2010). *A. hypogaea* forms effective nodules with *Bradyrhizobium* sp. (Urtz and Elkan, 1996; El-Akhal et al., 2008; Ngo Nkot et al., 2008; Chen et al., 2014), but data on nitrogen fixation efficiency and taxonomic diversity of rhizobial strains in peanut fields in Senegal are scarce (Sene et al., 2010). Thus, understanding the process that favoured

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peanut adaptation in Senegal by mining the extent of the diversity of the native strains found in peanut nodules and their efficiency in fixing atmospheric nitrogen is an important step to identify elite strains that can be used as potential inoculants to improve peanut productivity in Senegal through BNF.

In this work, we studied the phenotypic and genetic diversity of rhizobia isolated from *A. hypogaea* grown in different agroecological zones in Senegal and compared these strains with efficient strains from Argentina and Zimbabwe.

2. Material and methods

2.1. Sampling sites

This study focused on three agro-ecological zones of Senegal (Fig. S1): the Coastal zone, the Sylvopastoral area and the Groundnut basin. The Coastal zone is located in the western part of the country and runs along the Atlantic coast. Fruits and vegetables are produced in this region during the dry season. The average rainfall rarely exceeds 1000 mm. The Sylvopastoral area is located in the northern part of the country and corresponds to a Sahelian climate with a rainfall generally below 600 mm. The Groundnut basin is the main peanut growing area and is mainly located in the Sahelian Sudanese and Sudanian climatic domain with a mean rainfall between 500 and 1200 mm.

2.2. Soil sampling

Sixty-eight soil samples were collected from 53 different locations during the dry season. Soil have been sampled to a 20-cm depth from fields which have no history of peanut inoculation.

2.3. Plant material

Experiments were conducted using the Fleur11 cultivar of *Arachis hypogaea* L. Ssp. Fastigata. Fleur11 is an improved cultivar, Spanish type, high yielding with short cycle (90 days). The cultivar is one of the parents of several interspecific (cultivated x wild) populations and widely grown in the Senegalese Groundnut basin and other West-African countries. (Clavel et al., 2005; Fonceka et al., 2012; Nguepjop et al., 2016).

2.4. Bacterial strains

2.4.1. Reference strains

Three strains able to nodulate peanut: *Bradyrhizobium* sp. SEMIA6144 which was isolated in Zimbabwe (Germano et al., 2006) and two efficient strains isolated in Argentina: *Bradyrhizobium* sp. CH81 and *Bradyrhizobium* sp. LH237 (Muñoz et al., 2011; Valetti et al., 2016)

2.4.2. Trapping experiment to isolate indigenous strains

A. hypogaea seeds were sterilized with 1.81% calcium hypochlorite for 8 min, washed five times with sterile distilled water and germinated in the dark for 72 h at 28 °C in Petri dishes containing 0.9% agar. The seedlings were sown in 11-cm diameter pots by containing 1 kg of each of the sampled soils and one seedling was grown per pot. The pots were placed in a shade house in a completely randomized block design with three replicates. Plants were watered daily.

Plants were harvested at 40 days after planting. Five nodules were randomly collected per plant and bacteria were isolated from nodules on YEM medium supplied with 0.002% Bromothymol blue as described by Andrés et al. (1997). Isolates were stored in 20% glycerol at −80 °C since 1990.

2.4.3. Infectivity of bacterial strains

The ability of the isolated strains to induce nodule formation in *A. hypogaea* was first tested in semi-axenic conditions as follows: seeds were surface sterilized and pre-germinated as described above. After germination, seedlings were transferred to glass tubes containing a sterile nitrogen-free Jensen solution (Jensen, 1942) as described by Vincent, (1970). Bacterial cultures were grown at 28 °C in YM (yeast extract and mannitol) liquid medium for 2–5 days until absorbance measured at 600 nm reached 0.6. The bacterial cultures were centrifuged at 6,480 × *g* for 5 min at 10 °C and resuspended in phosphate-buffered saline buffer (Sambrook and Russell, 2001). For 4 mL of bacterial cultures, the centrifuged bacterial cells are resuspended in 4 mL of saline buffer. In each tube, 1 mL of bacterial suspension (corresponding approximatively to 10^8 bacterial cells) was used to inoculate seedling radicles. Four replicates were done for each strain. The experiment was carried out in a growth chamber at 28 °C with a 16 h photoperiod at 54 µmol $m^{-2} s^{-1}$. Plants were harvested 28 days after planting and the number of nodules was visually scored. Non-inoculated plants were used as negative controls.

2.4.4. Molecular analysis

Total genomic DNA was isolated from 1 mL of a bacterial culture using the blood genomic-Prep Mini Spin Kit (GE Healthcare). The intergenic spacer (IGS), corresponding to the region between 16S and 23S rDNA and the symbiotic gene *nodC* were amplified using the primers listed in supplemental Table S6. PCR was performed in an Applied Biosystems 2720 thermocycler using the following program for both the IGS and *nodC* gene: an initial denaturation at 94 °C for 5 min followed by 35 cycles at 94 °C for 30 s, 55 °C for 30 s, and 72 °C for 1 min, and a final elongation step at 72 °C for 7 min.

Samples were sequenced by Genoscreen (Lille, France). Nucleotide sequences were edited manually using the software SeqMan II version 8.0 (DNASTAR, Wisconsin, WI, USA). The closest sequences were retrieved from GenBank using a BLASTN search (Altschul et al., 1990). IGS sequences corresponding to strains isolated from *Arachis* were also retrieved from GenBank using a keyword search. The IGS sequences described in Grönemeyer et al., 2016 and Muñoz et al., 2011 were also included. Maximum likelihood phylogenies were constructed using the phylogeny.fr platform (Dereeper et al., 2008) applying the default settings, except for GBLOCKS, which was configured to be less stringent. The bootstrap support for each node was evaluated with 500 replicates. All sequences were submitted to NCBI and accessions number are available in Table S7.

2.5. Setting up an experimental system to evaluate symbiotic phenotypes

To assess the symbiotic phenotype of peanut symbionts a growth substrate was obtained by wet-sieving (2 mm) a Loam in three changes of water each of 2.4 L as to prevent inhibition of nodulation by excess soil Nitrogen (Streeter) (Streeter, 1985). The locally available commercial substrate that we used in this study contained 65% of organic matter and chemical fertilizers. Peanut seeds were surface sterilized and germinated as described above. After germination, the seedlings were transferred to 800 mL pots containing 200 g of leached-out loam sterilized at 120 °C for 20 min. The pots were previously disinfected with a solution containing 1.81% of calcium hypochlorite. A total of 45 plants were transplanted and 39 young plants were inoculated three days after seedling transplantating by adding 5 mL of a SEMIA6144 suspension prepared as above. Six young non-inoculated plants were used as negative controls. The plants were cultivated in a shade house and watered every two days. We used a completely randomized design, with 3 repetitions per treatment (inoculated and uninoculated). From the 4th day post inoculation, three plants were harvested every two or three days and the number of nodules per plant was counted. Plants were harvested after 45 days post inoculation. The effectiveness of N_2 fixation was thus evidenced by observing the presence of leghaemoglobin (pink color) inside dissected nodules using a stereomicroscope; nodules showing leghaemoglobin accumulation were considered effective. The percentage of effective nodules was determined on each plant. Experiments performed with this substrate showed that at the 5th week after the inoculation all the plants inoculated with the reference strain SEMIA6144 had developed effective nodules. We therefore started measuring

all parameters related to symbiosis efficiency at this time point in the following experiments.

2.6. Evaluation of plant responses to inoculation

Preliminary experiments performed with this substrate showed that at the 5th week after the inoculation all the plants inoculated with the reference strain SEMIA6144 had developed effective nodules. We therefore started measuring all parameters related to symbiosis efficiency at this time point in the following experiments. The performance of the rhizobia strains was evaluated using peanut cultivar Fleur11 as the host by the summary of previous section as described above. The symbiotic performance of each Senegalese native rhizobial strain was evaluated. We also included the reference strain SEMIA6144 (Germano et al., 2006), two efficient strains isolated and well characterized in Argentina (Muñoz et al., 2011), a non-inoculated negative control and a positive non-inoculated control fertilized with urea. Plants were cultivated for 7 weeks in sterilized leached-out loam and under controlled shade house conditions. The experimental design was a completely randomized block with three replicates. Positive (uninoculated and fertilized with urea) and negative (uninoculated and unfertilized) controls were included. The positive controls received 1.2 g of urea per plant at the beginning of the experiment. The plants watering was performed every two days. The relative chlorophyll content of leaves was measured once a week with a SPAD-502 chlorophyll meter (Konica-Minolta). Root dry mass (Rdm), shoot dry mass (Sdm), and nodule dry mass (Ndm) were recorded on plants harvested seven weeks after inoculation. Total number of nodules (NNbr) was counted using the WinRHIZO software (Diener et al., 2013). The total leaf chlorophyll content (Total Chl.) was quantified based on absorbance as described by Lichtenthaler and Wellburn (1983). A multiple comparison was performed using the mutlicom package using the R statistical software. The mean responses of inoculated plants (Rdm, Sdm and Chlorophyll) were compared to the non − inoculated control using the Dunnett test. A principal component analysis (PCA) was made using biomass, nodule and chlorophyll data with the ADE4 and ADE4TKGUI packages using the R statistical software. The PCA plot was formatted to visualize the three clusters (A, B and C) defined from the IGS and the number of nodules obtained after inoculation by each strain.

3. Results

3.1. Starting a senegalese peanut rhizobia collection

To establish a collection of Senegalese rhizobia able to nodulate peanut we collected soil samples at 53 different sites located in three agroecological zones in Senegal (Fig. S1) and used peanut as a trap host. We obtained a total of 144 isolates. Eight strains were obtained from the Coastal zone, 27 from the Sylvopastoral zone and 109 from the Groundnut basin (Table S5). Thirty-five strains were able to induce nodules (either effective or not effective) under semi-axenic conditions. Hereafter these 35 strains are referred to as native strains. Effective nodules were also obtained from plants inoculated with the three reference strains. No nodulation was observed the non-inoculated negative controls.

3.2. Genetic diversity of the Senegalese peanut rhizobia collection

The 35 native strains were genetically characterized using total DNA as a matrix to amplify the 16S/23S intergenic region (IGS) and the *nodC* gene. The sequences obtained after IGS sequencing were used to build a maximum-likelihood (ML) phylogenetic tree including the closest sequences found in GenBank, strains previously isolated from *Arachis* sp. and reference *Bradyrhizobium* strains (Fig. 1). Sequences obtained from the native strains shared between 95% and 100% identity with *Bradyrhizobium* sequences. Three main clusters with well supported bootstrap values were named A, B and C (Fig. 1). Five subgroups were defined in group A. Subgroup A1 contained two reference strains (SEMIA6144 and CH81), the *B. arachidis* type strain but no native strains. Subgroup A2 contained two native strains, ISRA519 and ISRA524, which are close to *B. yuanmingense*. ISRA519 was isolated in soil from the 'Groundnut basin' and ISRA524 in a soil from the 'Sylvopastoral zone'. Several peanut strains from Asia and America were found within or

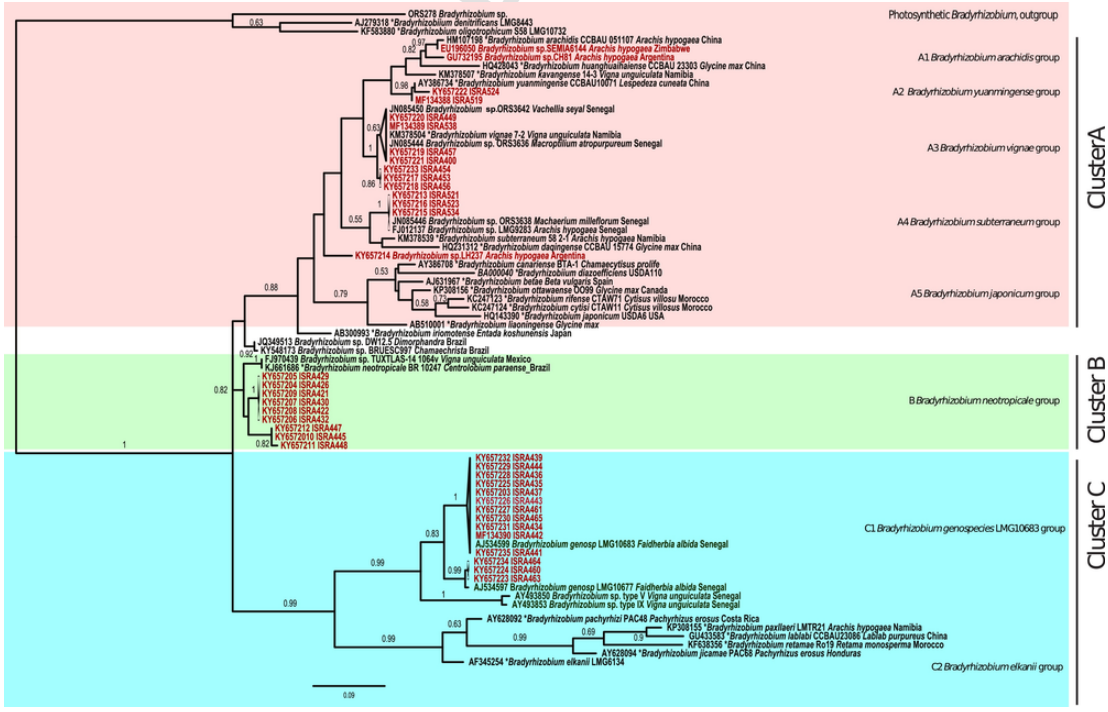

Fig. 1. Maximum likelihood phylogeny constructed with 1260 bp sequence fragments corresponding to the 16S-23S rRNA intergenic spacer sequences. ML analysis was performed using the phylogeny.fr pipeline on a dataset including sequences generated in this study, the closest sequences retrieved from GenBank and reference strains described in Grönemeyer et al., 2016 and Muñoz et al., 2011. The strains used in this study are in red. Bootstrap values above 0.5 for 500 replicates are indicated. When known, host species names and geographic origin are indicated after the strain name. *: type strain.

close to subgroups A1 and A2 (Fig. S2). The third Subgroup, A3, contained seven native strains which clustered together with *B, vignae* isolated from cowpea and two strains isolated from the African trees *Vachellia seyal* and *Macroptilium atropurpureum*. Native strains belonging to this group were all isolated of soil from the Groundnut basin. Only one peanut strain previously isolated in Namibia belonged to this group (Fig. S2). Sub-cluster A4 contained three native rhizobia from the Groundnut basin and strains isolated from nodules of *Machaerium milleflorum* (ORS33638), the strain LMG9283 isolated previously from peanut in Senegal and the *B. subterraneum* type strain isolated from peanut in Namibia. Native strains were not represented in sub-clusters A1 and A5 that contained numerous strains isolated from *Arachis* sp. in Asia, America and Africa (Fig. 1; Fig. S2).

Cluster B contained nine native strains and two strains isolated from tropical trees in the Americas including the *B. neotropicale* type strain BR10247 (Fig. 1). Native strains belonging to cluster B were isolated from the Groundnut basin and from the Coastal zone. Interestingly, cluster B did not contain any of the strains previously isolated from *Arachis* sp. (Fig. S2).

The remaining native strains belonged to the well supported C cluster that contained two sub-clusters, C1 and C2. Native strains were only found in sub-cluster C1, which also contained two strains isolated from *Faidherbia albida,* a tree very often found growing close to Senegalese peanut fields, and two strains isolated from cowpea, also grown in Senegal (Krasova-Wade et al., 2003). C2 was the sister group to C1 and contained the *B. elkanii* type strain and several strains isolated from *Arachis* sp. in Africa, Asia and America. (Fig. 1, Fig. S2).

To further characterize the isolates at the genetic level using genes directly involved in the nodulation process, we also constructed phylogenies based on the *nodC* gene that encodes a chitin synthase involved in the initial step of Nod factor synthesis (Lloret and Martínez-Romero, 2005; Mergaert et al., 1997) (Fig. 2). Thirty-seven sequences were obtained using *nodC* primers (Table S6). Results revealed seven well supported the clusters named N1 to N7, of which five contain native strains. Clusters observed based on *nodC* phylogeny (Fig. 2, Fig. S3) showed a high similarity to those based on sequence analysis of the IGS (Fig. 1, Fig. S3) with the notable exception of two native strains (ISRA432 and ISRA456) that belonged to different groups in both IGS and *nodC* phylogenies.

3.3. Characterization of symbiotic phenotypes

In order to assess the nodulation efficiency of peanut symbionts we first developed an experimental system using leached autoclaved loam that allowed peanut plants to develop and nodulate under controlled conditions.

Using this system the symbiotic performance of each Senegalese native rhizobial strain was evaluated. We also included the reference strain SEMIA6144, two efficient strains isolated and well characterized in Argentina (Muñoz et al., 2011), a non-inoculated negative control and a positive non-inoculated control fertilized with urea. We measured parameters related to plant growth: leaf and shoot dry mass (Sdm), root dry mass (Rdm), and relative leaf chlorophyll content. Nodules were not detected in unfertilized control and N fertilized controls. Plants inoculated with twenty native strains, the ref-

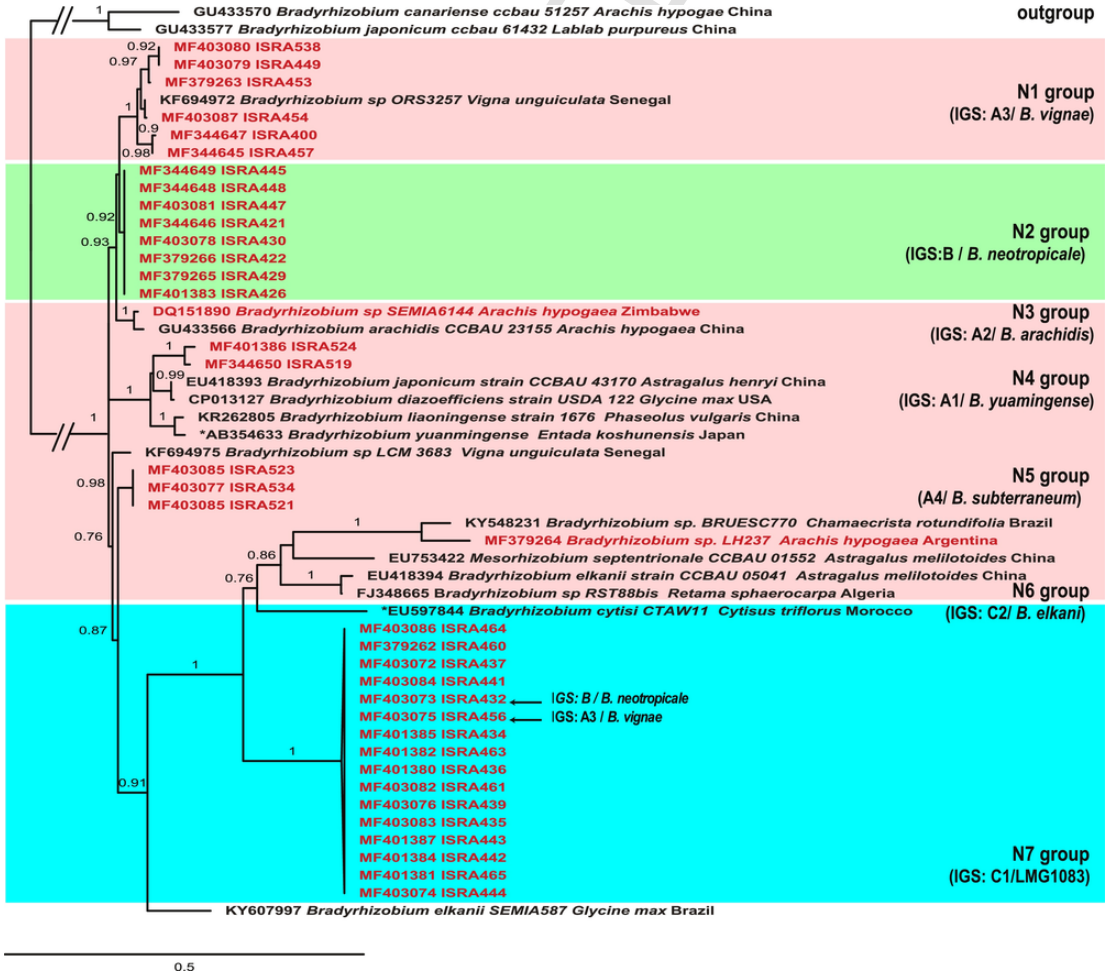

Fig. 2. Maximum likelihood phylogeny built using a 715 bp fragment of the nodC gene sequence. ML analysis was performed using the phylogeny.fr pipeline on a dataset including sequences generated in this study and the closest sequences retrieved from GenBank. Bootstrap values over 0.5 for 500 replicates are shown. When known, host genus names are indicated after the strains name. Strains used in this study are indicated in red.

erence strains and plants fertilized with urea showed significantly increased chlorophyll content (SPAD) compared to the negative control (Fig. 3). Strains showing the highest chlorophyll content (SPAD) belonged to clusters A and B as defined using the IGS phylogeny and only three strains from these groups showed chlorophyll levels similar to the negative control (Fig. 3). Among these 20 strains, 15 came from the Groundnut basin.

Six native strains (ISRA400, ISRA422, ISRA454 ISRA457, ISRA461 and ISRA534) and the reference strain CH81 showed significantly higher Sdm values than the non-inoculated control. These seven strains were also obtained from the Groundnut basin. Regarding root dry mass, only the reference strain CH81 showed a significant increase compared to the negative control. Overall, 57% of the native strains used in this study were able to improve at least one measured parameter. All these native strains, except ISRA426, ISRA429, ISRA443, ISRA464, and ISRA524 originated from the Groundnut basin soils.

3.4. Analysis of relationships between the symbiotic performance and the genetic diversity of strains

Table 1 shows the quantitative classification and frequency of nodulation. Differences of several logs were observed between the least nodulated and the most nodulated plants (Table 1; Fig. S4). The highest number of nodules was found in the Argentinian reference strain CH81, which induced more than 1100 nodules per plant, followed by the native ISRA441 (1050 nodules). Forty-seven percent of the native strains induced between 100 and 300 nodules per plant. The reference strains LH237 and the SEMIA6144 and those producing a significant increase in shoot dry mass (except for ISRA461) induced fewer than 300 nodules per plant. With the exception of ISRA524, all native strains that induced more than 300 nodules belonged to cluster C defined based on the IGS phylogeny (Table 1, Fig. 1) and plants inoculated with those strains showed no significant increase in Sdm, Rdm or relative leaf chlorophyll values (Fig. 3; Fig. S4). Taken together these results suggest that efficient associations with native strains occurred generally in plants that developed few nodules with strains belonging to clusters A and B.

Principal component analysis (PCA) to assess plant response to inoculation was performed using the 35 native Senegalese strains (Fig. 4). The first two axes, PC1 and PC2, explained 67% of the total variance. The responses to inoculation made it possible to distinguish the native strains according to their phylogenetic groups defined by the IGS phylogeny. When information on the number of nodules was superposed to the PCA analysis, we found that the PC1 axis was very strongly correlated with the number of nodules induced by each strain. Strains belonging to group A were also those that formed the lowest number of nodules (less than 500). Under PCA analysis (Fig. 4), native strains corresponding to the highest relative leaf chlorophyll were generally grouped with those that induced fewer nodules (less than 500) and only five of the 20 native strains were able to improve Sdm. On the other hand, ISRA457 and ISRA461 induced a significant increase in plant Sdm but did not affect the chlorophyll content.

4. Discussion

4.1. Infective strains isolated from peanut nodules are close to Bradyrhizobium species often found in native plant species

This is one of the first reports on the genetic diversity of peanut rhizobia symbionts isolated in Senegal. By screening 144 strains originally isolated in the 1990s from peanut nodules sampled in different agroecological zones of Senegal, we showed that only 35 of them were infective on peanut. Many strains were not able to induce the formation of nodules; these were either endophytic or opportunistic bacteria trapped together with *Bradyrhizobium* but unable to induce nodule formation on their own. Endophytic bacteria inhabit the nodules jointly with rhizobia but their role in nodulation is still poorly known (Ibáñez et al., 2009; Velázquez et al., 2017). The ability of some strains to induce nodules could also be linked to suboptimal storage conditions (Calcott, 1986).

Phylogenetic analysis based on the 16S/23S intergenic regions of infective strains showed that the 35 strains belonged to the genus *Bradyrhizobium* based on a high similarity (95–100%) to sequences in GenBank. This result confirms

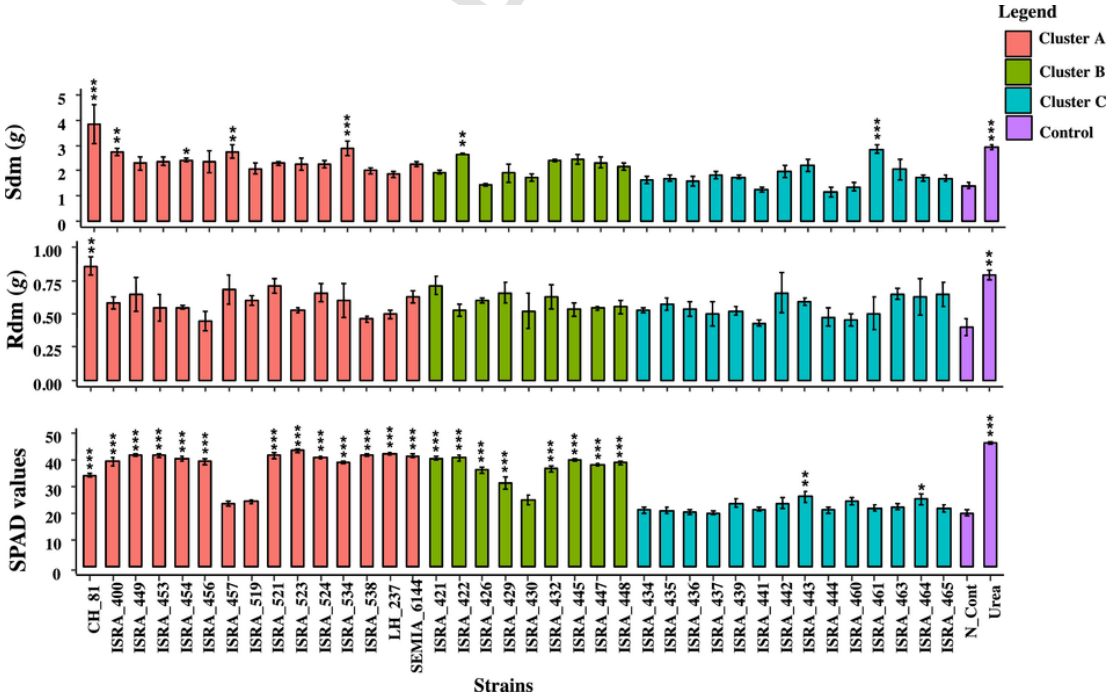

Fig. 3. Phenotypic characterization of native strains. The effect of 35 native strains and of 3 reference strains Bradyrhizobium sp. SEMIA6144; Bradyrhizobium sp. CH81 and Bradyrhizobium sp. LH237 was evaluated in greenhouse conditions 7 weeks after inoculation. Upper chart: Shoot dry mass per plant (Sdm); middle chart: Root dry mass per plant (Rdm); Lower chart: Relative chlorophyll content (SPAD: Soil Plant Analysis Development). The stars above the bars show a significant difference compared to the negative control (ANOVA + Dunnett's test, *P < 0.05;**P < 0.01; ***P < 0.001). Bars indicate the standard error.

Table 1
Quantitative distribution of nodulation (parameter: number of nodules) in the frequency classification table. Data represent the effect of 35 native strains and the 3 reference strains: Bradyrhizobium sp. SEMIA6144; Bradyrhizobium sp. CH81 and Bradyrhizobium sp. LH237.

Strains	Class Interval of NNbr	Frequency
ISRA449, ISRA453, ISRA534, SEMIA6144, ISRA400, ISRA454, ISRA448	[0–100[	7
ISRA447, ISRA445, ISRA519, ISRA538, ISRA457, ISRA460, ISRA523, LH237, ISRA421, ISRA432, ISRA430, ISRA464, ISRA422, ISRA456, ISRA434, ISRA429, ISRA521, ISRA426	[100–300[	18
ISRA524, ISRA463	[300–500[	2
ISRA461, ISRA435, ISRA465, ISRA436	[500–700[	4
ISRA437, ISRA444, ISRA439	[700–900[	3
ISRA442, ISRA443, ISRA441	[900–1100[	3
CH81	[1100–1300[	1

that *Bradyrhizobium* is the major root nodule symbiont of peanut (Muñoz et al., 2011; Degefu et al., 2012; Chen et al., 2014). Although some fast growing species such as *Rhizobium giardinii, R. tropicii*, *R. huautlense* and *Rhizobium galegae* have been found associated with peanut in Argentina and Morocco (Taurian et al., 2006; El-Akhal et al., 2008), these types of rhizobia were not present among the Senegalese native strains described here. Phylogenetic analysis also revealed the presence of a new subgroup of *Bradyrhizobium* forming a symbiotic association with peanut (cluster B containing *B. neotropicale*). Novel genospecies of *Bradyrhizobium* that nodulate peanut have been described in many countries including Angola, Namibia, Botswana and China (Chen et al., 2016; Grönemeyer et al., 2016). Well supported groups obtained based on the IGS sequences were also retrieved using the *nodC* gene, suggesting that the *nodC* and IGS regions are generally transmitted vertically. The few exceptions we found here, point to occasional lateral gene transfers between native *Bradyrhizobium* species, which has already been described for symbiotic genes like *nodC* (Laguerre et al., 2001; Moulin et al., 2004; Mutch and Young, 2004; Vinuesa, 2005; Barcellos et al., 2007).

Clusters A and C contained many strains previously isolated from peanut nodules, but interestingly, native strains belonging to these two groups were found to be close to bacteria previously isolated from the roots of *Macroptilium atropurpureum, Machaerium milleflorum, Vachellia seyal, Faidherbia albida* and cowpea, five legume species that are commonly found in farmers' fields. Also interestingly, several groups of native peanut strains, such as A3 or C1, contained no strains of American origin, suggesting that peanut was either introduced in Senegal without its original symbionts or that the original symbionts were outcompeted by local strains that nodulate native species. The recruitment of these strains was probably favoured by cultural practices that are common in Senegal, such as agroforestry, crop association or rotations that enable close contact between peanut plants, legume trees and other legume crops. Our results are in agreement with those of previous studies showing that peanut can be nodulated by *Bradyrhizobium* isolates from *Glycine max* (Yang and Zhou, 2008), or *Vigna unguiculata* (Krasova-Wade et al., 2003) thereby confirming that peanut is nodulated by a broad range of *Bradyrhizobium* species.

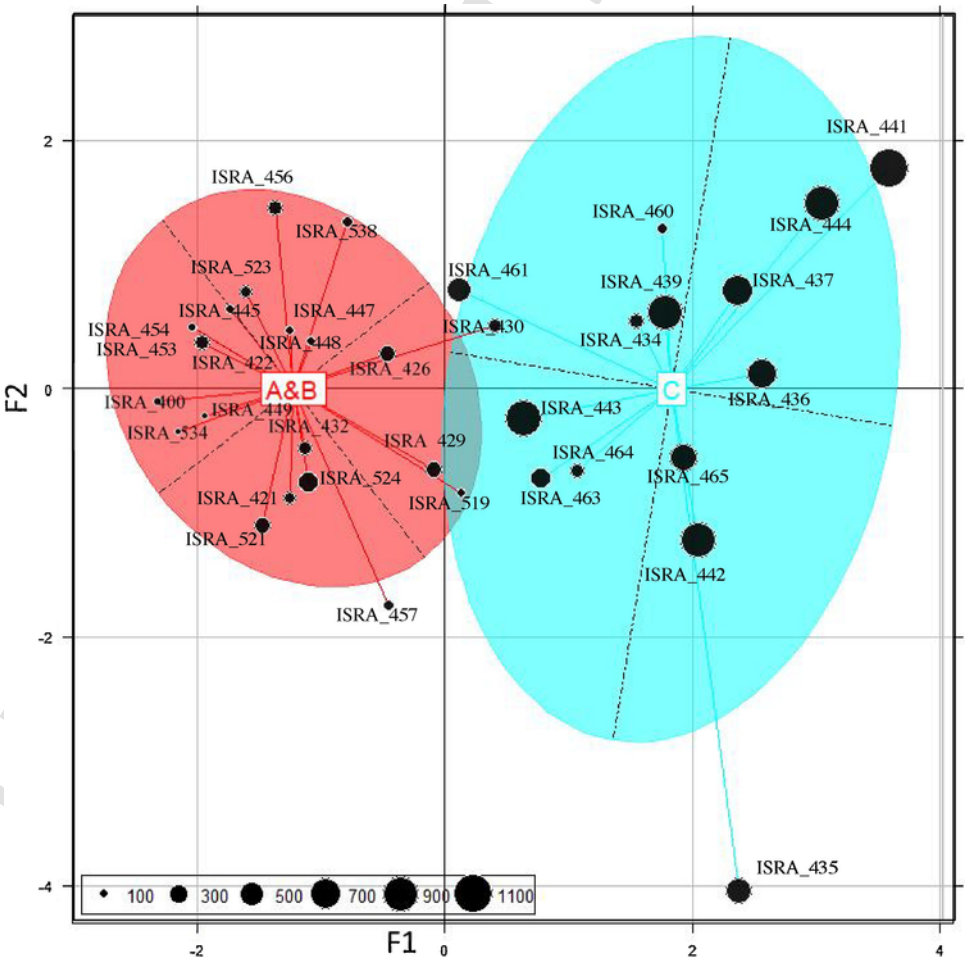

Fig. 4. Principal component analysis showing the relationships between the number of nodules; shoot dry mass (Sdm); root dry mass (Rdm); total leaf chlorophyll (Total Chl.) and relative chlorophyll content (SPAD) of 35 peanut strains isolated from different agroecological zones in Senegal. The size of the circle is proportional to the number of nodules.

4.2. The most efficient strains come from the groundnut basin

In addition to the genetic diversity, we analysed the N2 fixation potential of several strains base on chlorophyll content, root and leaf dry mass and the number of nodules in peanut plants inoculated with native strains or reference strains compared to non-inoculated plants or plants fertilized with urea. Most of the rhizobia exhibiting efficient symbiotic performance come from the Groundnut basin. Many studies have shown the variations in the functional diversity of strains is influenced by the environmental conditions in their zone of origin (Cardoso et al., 2009; Degefu et al., 2012; Mokgehle et al., 2014). The high proportion of efficient strains originating from the Groundnut basin could be explained by the high number of soil samples analysed from this area, and by the fact that this zone has a long tradition of food crop and peanut cultivation.

4.3. Efficient native strains produce few nodules and generally do not belong to cluster C

With the exception of CH81, the plants that developed a large number of nodules were those inoculated with strains belonging to cluster C, which also showed poor symbiotic efficiency measured by chlorophyll levels or Sdm. Low chlorophyll levels reflect nitrogen starvation, which is known to stimulate nodule formation (Fei and Vessey, 2009). Interestingly, the inefficient nodules formed by most cluster C strains were unable to provide enough nitrogen to the plant, consequently stimulating the plant to form new nodules, but their formation apparently did not trigger the autoregulation of nodulation (AON) mechanisms, which inhibit the formation of new nodules in other legume species (Mortier et al., 2012). More detailed studies would be needed to investigate to what extent AON mechanisms described in other legume species are present in *Arachis sp*. Numerous ineffective nodules probaly draw signifficant amounts of energy with a potential negative impact on plant growth (Werner et al., 1980; Vance and Johnson, 1983; Eardly et al., 1985).

4.4. Future improvement of peanut symbiosis in Senegal

Field experiments in Argentina showed that inoculation with elite rhizobia resulted in a 44% increase in pod yield compared to that of the non-inoculated plants (Valetti et al., 2016) and rhizobia inoculum specially developed for peanut can be purchased from several companies. The identification of rhizobia strains associated with peanut in Senegal and their phenotypic characterization provides important background information for the development of peanut inocula suited for Senegalese fields. Local strains that are more adapted to local conditions could probably be more competitive than foreign strains. Experiments incorporating soils containing native rhizobia populations under controlled and field conditions are necessary to assess the competitiveness of effective N2 fixing strains identified in this study. However, the effective strains identified in this study could potentially be used to inoculate peanut in other sahelian countries with similar characteristics like subhumid, semi-arid and arid climates.

Peanut productivity can be enhanced by improved symbiosis with *Bradyrhizobium* strains. In this regard, we are currently using efficient Senegalese strains described above to compare the symbiotic effectiveness of peanut cultivars from Senegal and to map QTLs related to nitrogen fixation efficiency in mapping populations developed at CERAAS (Fonceka et al., 2012).

Uncited references

Navarro et al. (1992), Ponsonnet and Nesme (1994) and Sarita et al. (2005).

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.agee.2018.06.001.

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