



Eco-geographical diversity of cowpea bradyrhizobia in Senegal is marked by dominance of two genetic types[☆]

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ABSTRACT

The genetic diversity of native cowpea rhizobia originating from 60 sites across four eco-geographic zones in Senegal was studied. More than 300 cowpea nodules were analyzed by PCR-RFLP of the 16S–23S rDNA InterGenic Spacer region (IGS). Alignments of IGS sequences indicated that all genotypes were grouping within the *Bradyrhizobium* genus. The geographical distribution showed that apart from five IGS types, the others were specifically found in only one region. The diversity was significantly higher in the Senegal River valley zone, which presents lower mean annual rainfalls and slightly alkaline soils. Interestingly, two IGS types dominated the Senegalese rhizobial collection, one IGS type (VI) was found on more than half of the nodules collected in the northern Senegal River valley while another IGS type (I) was recovered from the great majority of nodules in the three other regions sampled. Two representative strains from each of these two dominant types were isolated and further analyzed. Multi Locus Sequence Analyses using 6 housekeeping genes indicate that they belong to a new *Bradyrhizobium* species closely related to *B. yuanmingense*. Phylogenetic analyses of 2 symbiotic genes *nodC* and *nifH* show that they are clustered with *B. arachidis*. Physiological tests on these strains have shown that under laboratory conditions, the growth of the IGS type VI strains was slightly less affected by a higher osmotic strength in the medium and to alkaline pH, which corroborates the soil physico-chemical parameters.

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[☆] Note: Nucleotide sequence data reported are available in the GenBank database under the accession numbers: for the 16S–23S rDNA IGS of representatives of the IGS types V, VI, VII, VIII, IX, X, XI, XII, XIII, XVII, XIX and XX, respectively, AY493850, AY493849, AY493851, AY493852, AY493853, AY493854, AY493855, AY493856, AY493857, AY493858, AY493859, AY493860; accession numbers for IGS 16S–23S rDNA of the strains ORS 3258, LCM 3682, LCM 3683 are KF697400, KF697401, KF697402. Nucleotide sequences of housekeeping genes from the strains ORS 3257, ORS 3258, LCM 3682 and LCM 3683 are available, respectively, under accession numbers JN225382-5 for *atpD* gene; JN225387-9 and JN225390 for *dnaK* gene; JN225392-5 for *gyrB* gene; JN225397-9 and JN225400 for *recA* gene; JN225402-5 for *rpoB* gene; KF694980-3 for *glnI* gene, and of symbiotic genes, are KF694972-5 for *nodC* gene; KF694976-9 for *nifH* gene.

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Introduction

Cowpea (*Vigna unguiculata* (L.) Walp.) is one of the most important, versatile, and nutritious grain legume in semi-arid regions of the tropics covering 14×10^6 ha across Asia, Africa and South America [48]. More than 80% of the world production comes from West Africa [23]. Cowpea is emerging as one of the most important food legume in semi arid regions because of its early maturity and due to its possible use in multiple cropping systems [49]. This crop is commonly grown on small scale by ill-resourced farmers and do not benefit from commercial companies to provide seeds [24]. Despite the nutritional qualities of cowpea, the yields in African countries

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remain low with 300–400 kg/ha [23,48]. Even though this legume is considered as relatively drought tolerant, frequently mentioned causes for its limited yields include environmental constraints such as drought, low soil fertility or low nitrogen fixation efficiency of indigenous rhizobia to supply the plants with optimal nitrogen levels through symbiotic associations [13]. Furthermore, a recent study by Pule-Meulenber et al. [41] showed that cowpea growth depends on N₂ fixation which underlies the need to study the diversity, competitiveness, efficiency and the physiology of its associated symbiotic rhizobial partners.

Different environmental factors have been reported to affect indigenous rhizobia competition for nodule occupancy [19,40,52,55,57,64], but the mechanisms controlling the distribution and abundance of soils microorganisms are still poorly understood [45]. Assuming that nature shapes the organisms diversity, there are many environmental factors and multiple levels (from micro niches to regional climatic constraints) to which particular bacteria are adapted. Some of these adaptive mechanisms may contribute to survival and infectiveness of indigenous rhizobial populations [52].

Senegal provides an excellent opportunity to study the cowpea-rhizobia interactions in relation to the environmental constraints. Ranging from desert in the north (Sahelian zone) to tropical in the south (Soudano-Guinean zone), the pedo-climatic factors are highly variable across the Senegalese territory. Despite the heterogeneous physico-chemical properties of the soils and the contrasted climate encountered across Senegal, cowpea is cultivated throughout the country during the rainy season (from July to November). This legume crop is, however, principally grown in the north (dry steppe, receiving irregular rainfalls of 100–300 mm

per year) or in the center (savannah, with 400–800 mm per year) of the country (>90% of cowpea cultivated areas), the south (deciduous forest, with 800–1000 mm of rainfall per year) representing less than 10% of the cowpea cultivated area [12].

Earlier studies on the diversity of bradyrhizobia nodulating three cowpea cultivars subjected to different water regimes were performed at one field location in central Senegal [26,27]. RFLP analyses of the 16S–23S rDNA IGS (Inter Genic Spacer region) obtained from bacterial DNA extracts of 85 nodules and IGS sequence analyses showed that cowpea rhizobia belonged to at least four different *Bradyrhizobium* groups which could be assigned to three distinct genospecies. More than three quarters of the analyzed nodules harbored the same IGS type and the proportion of each IGS type was depending on both the water treatment and the cowpea cultivar and suggested that nodulating strains were more diverse in water-limited conditions.

With the goal to estimate the natural cowpea bradyrhizobia diversity in relation to environmental parameters in Senegal, a countrywide diversity study was conducted. Nodules were collected in sites covering all regions where cowpea is cultivated and spanning four of the six eco-geographic classically recognized zones in Senegal: Senegal River valley, Peanut basin, Eastern Senegal and Natural region of Casamance. The main goals of this study were (i) to describe the diversity of cowpea rhizobia, as assessed by PCR-RFLP, sequencing analyses of 16S–23S rDNA IGS along the eco-geographic zones in Senegal, (ii) to identify the taxonomy of the major types encountered using MLSA and symbiotic genes on selected strains and (iii) to test whether the observed distribution was influenced by the major physico-chemical soil parameters and/or to the rainfall levels.

Table 1
Eco-geographic zones, sampling sites and geographic distribution of IGS 16S–23S rDNA types.

No. site	Site	IGS type (nodules number)	No. site	Site	IGS type (nodules number)
Peanut Basin zone (A)			Eastern Senegal zone (B)		
1	Chamine	I (3)	30	Botou	I (5)
2	Darou Thiaou	I (2)	34	Gouloumbou	I (1), II (3), VII (1)
3	exit Louga	I (5)	35	Gourel Bokar field 1	I (5), XIX (1)
4	Guede Gueye	IX (1)	36	Gourel Bokar field 2	I (5), XX (1)
5	Ker Abdou	I (4)	37	Hamdalah pont	I (3)
6	Khandiar	I (14)	38	Hamdallaï Diapaldi	I (8)
7	Lam-Lam	V (1)	39	Inna Dinna	I (4)
8	Macka Diop	II (1)	41	Kotiari	I (5), XIX (1)
9	Ndiakhate	III (1)	43	Sare Dela	I (3)
10	Ndoucoumane	I (3)	60	Hamdallaï Diapaldi	I (23), IX (2)
11	Ngalagne	I (11)	Senegal River valley zone (C)		
12	Parba Langa	I (2)	44	Bokhol	VI (3)
13	Sarr Paleine	I (2)	45	Coyel	VI (1)
14	Sa-Sarr	I (2), VII (1)	46	Diawdine	VI (2)
15	Serrer field 1	I (4)	47	Diery Gaya	VI (5)
16	Serrer field 2	I (4)	48	Edy	VI (4), XII (1), XVI (1) XVII (1)
17	Taiba Chaou	I (3)	49	Gadiobé	XI (2)
18	Thillpeul	I (5)	50	Garage Medina Baye	VI (5)
19	Thinou Mbarick	I (4)	51	Km 31 before Louga	VI (5)
20	Tylmakha	I (7)	52	Kogga	XII (1)
21	Baba Garage	I (11), V (1)	53	Mboltogne	I (1), XI (1)
22	Diamale Nbirkilane	I (3)	54	Mbumba	VI (2)
23	Diouroup	I (9)	55	Ndioum	V (4), VI (3), X (1), XIII (2), XIV (1), XV (1)
24	Keur Mbouki field 1	I (3)	56	Ndioum Wallo field 1	II (4), VIII (2)
25	Ndioudiouf	I (8)	57	Ndioum Wallo field 2	II (1), VIII (2)
26	Ndofane Masserign	I (2), XVIII (1)	58	Touldé Galle	VI (2), X (2), XI (1)
27	Sandiara	I (6)	59	Ndioum	V (1), VI (8), X (5), XV (1)
28	Sessene	I (4)			
29	Sikilo	I (6)			
40	Keur Mbouki field 2	I (20), IV (1)			
Natural zone of Casamance (D)					
31	Carrefour Manda field 1	I (8)			
32	Carrefour Manda field 2	I (6)			
33	Carrefour Manda field 3	I (6)			
42	Medina Touat	I (5)			

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