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Genetic diversity of rhizobia nodulating *Arachis hypogaea* L. in diverse land use systems of humid forest zone in Cameroon

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ABSTRACT

Peanut (*Arachis hypogaea*) is one of the most important legume cultivated in the humid forest zone of Cameroon. However, in this country the diversity of rhizobial strains that can nodulate peanut was poorly understood. Forty-two strains from *Arachis hypogaea* were examined by restriction fragment length polymorphism (RFLP) analysis of 16S–23S rDNA genes amplified by polymerase chain reaction (PCR). A considerable level of genetic diversity was determined among those peanut *isolates*. Eight composite genotypes were obtained from the combined data of the RFLP analysis with four endonucleases. A correlation between land use system and the diversity of peanut isolates was observed in the current study. The highest diversity was found in the cocoa farm and fallow and the lowest one in peanut farm. Our results have shown that the genotype richness diversity of peanut isolates depends on the land use system.

1. Introduction

Peanut (*Arachis hypogaea* L.) is an important crop that provides food for direct human subsistence and other several food products. It takes a significant part in the economy of many countries in the world. In the humid forest zone of Cameroon, *Arachis hypogaea* is the major legume crop. The soil in this region is ferruginous and ferralitic, characterized by acidity and low fertility.

Legumes are usually nodulated by indigenous root-nodule bacteria. The symbiosis formed through interaction between rhizobia and legume plants, such as peanut, can transform atmospheric N_2 to ammonia thus supplying nitrogen to the plant and enhancing its ability to withstand stress, even in arid environments (Rodelas et al., 1999). The contribution of biological nitrogen fixation (BNF) was 40.9 kg/ha for *Arachis hypogaea* (Okito et al., 2004). However, in spite of the contribution of this legume to the soil, at the farm level, grain yields are often low and inconsistent. Inoculation of legume seed is an efficient and convenient way of introducing viable rhizobia to the soil and subsequently to the rhizosphere of legumes (Deaker et al., 2004).

Abundant types of indigenous rhizobia are widely distributed in various geographical and ecological areas of the world (Xu et al., 1995; Chen et al., 1997; Tan et al., 1999; Peng et al., 2002). Peanut has been reported to form effective nodules with slow-growing rhizobia (Van Rossum et al., 1995; Urtz and Elkan, 1996; Zhang et al., 1999; Saleena et al., 2001).

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Currently, Taurian et al. (2006) demonstrated that *Arachis hypogaea* L. is nodulated by *Bradyrhizobium* species and also by fast growing rhizobia closely related to *Rhizobium giardini* and *Rhizobium tropici* species.

Nodulation of peanut by indigenous bacteria is usually assumed to be adequate, and inoculation is seldom practiced. However, survival and effective functioning of *Rhizobium* populations are reduced by high soil temperatures, salt and osmotic stress, soil acidity and alkalinity, pesticide and fungicide applications as well as nutrients deficiencies stress (Zahran, 1999). Through inoculation of strains selected from indigenous populations, improved symbiotic efficiency can be achieved, which can lead to an increase in peanut yield and quality. Variations in the acidity tolerance of some Cameroonian strains of rhizobia were evaluated by Nwaga and Ngo Nkot (1998), but little is known about the genetic variation in peanut rhizobial populations indigenous to Cameroon. Knowledge about the indigenous population is necessary before the selection and application of the strains.

In Cameroon, four land use systems are mostly used among which mixed farming, fallow, cocoa farms and forest. The object of this study was to assess the genetic diversity of rhizobia isolated from *Arachis hypogaea* in different land use systems of Cameroon. This was done using RFLP analyse of the PCR amplified 16S–23S DNAr Inter Genic Spacer (IGS) region and host specificity.

2. Materials and methods

2.1. Bacterial strains

Rhizobia were isolated from soil samples in the laboratory using peanut as trapping host. Four different sites located in one agroecological zone of Cameroon, which have no history of *Arachis hypogaea* L. inoculation, were sampled. The rhizobia isolated and reference strains used in this study are listed in Table 1.

A total of 42 peanut-nodulation rhizobia isolates were obtained from the nodules of *Arachis hypogaea* in different land use systems of Cameroon using standard procedures (Vincent, 1970). Two strains described by Nwaga and Ngo Nkot (1998) are included. The purity of cultures was assured by repeated isolation from single selection. All strains were grown and maintained on yeast extract-mannitol (YM) with or without agar (Vincent, 1970).

Table 1 – Original host legume and the land use systems of rhizobia isolates used in this study

Land use systems	No.	Isolates	Host legume
Mixed farming[a]	1	AhBeC11	*Arachis hypogaea* L
Mixed farming[a]	2	AhBeC21	*Arachis hypogaea* L.
Mixed farming[a]	3	AhBeC31	*Arachis hypogaea* L.
Mixed farming[a]	4	AhBeC41	*Arachis hypogaea* L.
Mixed farming[a]	5	AhBoC11	*Arachis hypogaea* L.
Mixed farming[a]	6	AhBoC41	*Arachis hypogaea* L.
Mixed farming[a]	7	AhEC11	*Arachis hypogaea* L.
Mixed farming[a]	8	AhEC12	*Arachis hypogaea* L.
Mixed farming[a]	9	AhEC31	*Arachis hypogaea* L.
Mixed farming[a]	10	AhYC11	*Arachis hypogaea* L.
Mixed farming[a]	11	AhYC21	*Arachis hypogaea* L.
Mixed farming[a]	12	AhYC31	*Arachis hypogaea* L.
Mixed farming[a]	13	AhYC41	*Arachis hypogaea* L.
Mixed farming[a]	14	VUID1	*Vigna unguiculata* L.
Mixed farming[a]	15	VUXY1	*Vigna unguiculata* L.
Fallow	16	AhBeJ11	*Arachis hypogaea* L.
Fallow	17	AhBoJ21	*Arachis hypogaea* L.
Fallow	18	AhBoJ31	*Arachis hypogaea* L.
Fallow	19	AhBoJ41	*Arachis hypogaea* L.
Fallow	20	AhEJ21	*Arachis hypogaea* L.
Fallow	21	AhEJ31	*Arachis hypogaea* L.
Fallow	22	AhEJ41	*Arachis hypogaea* L.
Fallow	23	AhEJ42	*Arachis hypogaea* L.
Fallow	24	AhYJ11	*Arachis hypogaea* L.
Fallow	25	AhYJ41	*Arachis hypogaea* L.
Cocoa	26	AhBoP31	*Arachis hypogaea* L.
Cocoa	27	AhBoP41	*Arachis hypogaea* L.
Cocoa	28	AhEP11	*Arachis hypogaea* L.
Cocoa	29	AhEP21	*Arachis hypogaea* L.
Cocoa	30	AhEP31	*Arachis hypogaea* L.
Cocoa	31	AhYP21	*Arachis hypogaea* L.
Cocoa	32	AhYP31	*Arachis hypogaea* L.
Cocoa	33	AhYP41	*Arachis hypogaea* L.
Forest	34	AhBeF11	*Arachis hypogaea* L.
Forest	35	AhBeF21	*Arachis hypogaea* L.
Forest	36	AhBeF41	*Arachis hypogaea* L.
Forest	37	AhBoF32	*Arachis hypogaea* L.
Forest	38	AhBoF41	*Arachis hypogaea* L.
Forest	39	AhEF11	*Arachis hypogaea* L.
Forest	40	AhEF21	*Arachis hypogaea* L.
Forest	41	AhEF41	*Arachis hypogaea* L.
Forest	42	AhYF31	*Arachis hypogaea* L.

[a] Soil samples were collected during the peanut crop cycle.

2.2. 16S–23S DNAr PCR-RFLP

A couple of primer, 16S–23S primers designed from conserved regions of *Frankia* sp. rrn, FGPS1490-72 (located at positions 1490–1510 of the 16 S rDNA, 5′-TGCGGCTGGATCCCCTCCTT-3′) (Normand et al., 1996), and FGPL132-38 (located at positions 132-114 of the 23 S rDNA, 5′-CCGGGTTTCCCCATTCGG-3′) (Norman et al., 1992), were used for PCR amplification of 16S–23S rDNA IGS region. After having been washed two times, bacterial suspensions were used as template for the PCR amplification.

Polymerase Chain Reaction (PCR) was performed in 25 μl mixture containing 1.25 μl of each primer, ultra pure water 20.5 μl; 1.5 U of Taq polymerase (Ready-to-Go PCR beads, Pharmacia Biotech) and 2 μl of bacterial cells suspension. The temperature profile was as follows: initial denaturation at 94 °C fot 5 min; 35 cycles of denaturation at 94 °C for 30 s, annealing at 55 °C for 30 s and extension at 72 °C for 1 min; and a final extension step at 72 °C for 7 min.

Amplification was conducted in a thermocycler GeneAmp PCR System 2400 (Perkin Elmer), using the procedure described by Van Berkum et al. (1996). Restriction endonucleases, HinfI, HhaI, HaeIII and MspI were used to digest PCR products as specified by the manufacturer using and aliquot of 6 or 8 μl. The DNA fragments were separated electrophoretically on a 2.5% agarose Metaphor gel at 3.3 V cm^{-1} for 1 h. The gels were stained in aqueous solution of 1 mg ml^{-1} ethidium bromide