

*Full Length Research Paper*

# Variation in nodulation and growth of groundnut (*Arachis hypogaea* L.) on oxisols from land use systems of the humid forest zone in southern Cameroon

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**Improving biological nitrogen fixation through legume nodulating bacteria (LNB) inoculation requires knowledge on the abundance and effectiveness of indigenous population in the ferralsols. Nodulation of groundnut was examined under pots experiment in four location sites of the Humid-forest zone: Bertoua in the East; Ebolowa in the South; Bokito and Yaoundé in the Centre Regions of Cameroon and within each of the locations, in four land use systems (LUSs) of different levels of disturbance: mixed farming (1 to 3 years old); fallows (3 to 5 years old); cocoa plantation (> 20 years old); forest (> 30 years old). Results indicate that, soils under investigation are acidic with pH ranging from 3.68 in the Ebolowa forest to 6.92 in mixed farming at Bokito. Groundnut formed nodules in all the four LUSs. Soils from plantations and forests were poorly nodulated, whereas those from mixed farming and fallows were highly nodulated, with a positive and significant correlation ( $r \geq 0.406$ ;  $p < 0.0001$ ) observed between nodulation and plant biomass in all the studied sites. These results suggest a high variation in groundnut nodulating bacteria density in soils from diverse LUSs of the humid forest zone of Cameroon; although, there was a site effect.**

**Key words:** Acid soil, *Arachis hypogaea*, biomass, land use systems, multilocal, nodulation.

## INTRODUCTION

Many soils in the tropics are fragile, with high acidity and aluminium toxicity, high phosphorus fixation and low soil biodiversity (Cardoso and Kuyper, 2006). Nitrogen and phosphorus are the most limiting nutrients for crop production in these ferral soils (Dogbe et al., 2002). In Cameroon, acid soils cover more than 80% of arable lands (The, 2000). Synthetic fertilizers for improving soil

fertility are rarely available to most farmers. In addition, these fertilizers may induce soil acidification and become less efficient after many cropping years (Bado, 2002), leading to a high dependence of soil to N fertilizer for optimum yield (Fening and Danso, 2002). Since fallow practice to restore soil fertility is no longer possible because of land scarcity, there is a need of more efficient practices.

Four land use systems (LUSs) are common in humid forest zone in Cameroon: forest; cocoa or coffee plantation; fallow and mixed farming. Forest conversion to farms or plantations is increasing in response to popula-

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tion growth (Van Noordwijk et al., 2002). Cocoa (*Theobroma cacao*) plantation, is one of the dominant LUS, has served in the past as an economically-attractive land use but has been rejected by many farmers because of low and unpredictable prices on the world markets. Hence, farmers were forced to abandon cocoa or coffee plantations toward other income-generating opportunities such as food crops. Forest and some old plantations are used for annual crops such as groundnut in a mixed farming system. Under conditions of low external input, the use of biological nitrogen fixation appears to be a cheaper and environmentally friendly source of N for plants (Peoples et al., 1995). According to Bogino et al. (2006) a high degree of nodulation and nitrogen fixation in groundnut is provided by indigenous LNB in some soils from Argentina. Groundnut (*Arachis hypogaea* L.) is an important legume crop that provides food for human subsistence and other products. In Cameroon, groundnut is the major legume crop in slash and burn, traditional agricultural food production system. It is grown on 250,000 ha with more than half of the production area in the northern part of the country (Ntoukam et al., 1996). The yield is low, with an average  $0.85 \text{ t}\cdot\text{ha}^{-1}$  compared with  $1.05 \text{ t}\cdot\text{ha}^{-1}$  in Senegal and  $1.03 \text{ t}\cdot\text{ha}^{-1}$  in Nigeria (Anonymous, 2000). In sub-Saharan Africa, cropping systems, slash and burn agriculture and acid soil infertility could be major limiting factors for this crop yield. In addition, the density of indigenous LNB in diverse land use systems may affect nodulation and legume performances. According to Nwaga et al. (2010), land use changes and cropping practices such as burning may affect soil functioning in the humid forest, along with diversity and occurrence of beneficial micro-organisms. When comparing indigenous versus selected rhizobia from USA, yield diversity of 0.33 to 1.48 t/ha was noticed according to groundnut genotypes and 0.27 to 0.76 t/ha according to rhizobia x genotype interactions in northern Cameroon low rainfall regions (670-1068 mm) (Mekontchou et al., 2007). It is therefore, important to select acid LNB strains with high symbiotic effectiveness to improve legume production in tropical acid soils. One of the major problems to improve *A. hypogaea* in this region is 'flat pod phenomenon' or 'empty seeds phenomenon' of groundnut. Few data are available on the nodulation and plant growth of legumes such as *A. hypogaea* in the Congo Basin zone in Central Africa. Therefore, the objective of this study was to assess under greenhouse conditions, nodulation and plant growth of *A. hypogaea* on acidic soils from diverse land use systems in the humid forest zone of southern Cameroon.

## MATERIALS AND METHODS

### Study sites, experimental design and treatments

Experiments were carried out in soil samples (2 kg plastic bags) collected from four location sites of the humid forest zone: Bertoua in the East; Ebolowa in the South; Bokito and Yaoundé in the

Centre regions of Cameroon (Figure 1). Within each site, soils were collected in four replicates in the four different land use systems (LUSs): mixed farming, fallow (*Chromolaena odorata*), cocoa or coffee plantation and forest. The experimental design was thus, a split plot (4 x 4) x 4 with the 4 different land uses representing the main factors and the 4 study sites the sub-factors.

### Soil sampling and analysis

A total of 64 soil samples were collected in four location sites according to Swift et al. (2001). A composite soil sample was a mixture of ten sub-samples all collected from a LUS of the same location site. Aseptic precautions were taken during sampling and handling of each soil to avoid contamination. Soil sample were taken from sites that had no previous history of LNB inoculation. The 64 composite samples were taken from the top 0 to 20 cm soil depth, from which the un-decomposed plant material were removed by hand. The chemical analysis of the soil was conducted using standard methods (Anderson and Ingram, 1993).

### Assessment of nodulation and growth of *A. hypogaea*

Groundnut seeds of the A<sub>26</sub> variety were obtained from the Institute of Agricultural Research and Development (IRAD). Nodulation was assessed by growing germinated seed in perforated plastic bags containing more than 2 kg of soil samples. Seeds were surface sterilized in 3.3% calcium hypochlorite solution for 3 min and washed thoroughly with sterile water, re-suspended in 70% alcohol for 3 min and washed thoroughly with sterile water. The seeds were incubated to germinate in sterile Petri dishes containing 0.9% (w/v) water agar for 2 to 3 days at 28°C. After germination, six seedlings were sown in each plastic bag. Two weeks after germination, the number of plants was thinned to four per plastic bag, thus, sixteen per treatment.

Plants were watered and harvested 6 weeks after sowing. The root systems of individual plants were washed separately, their nodules picked and counted from sixteen plants per treatment. Plant shoot and roots systems were let to dry at 70°C for more than 72 h in an air dry oven and then weighed until constant mass (Athar and Johnson, 1996).

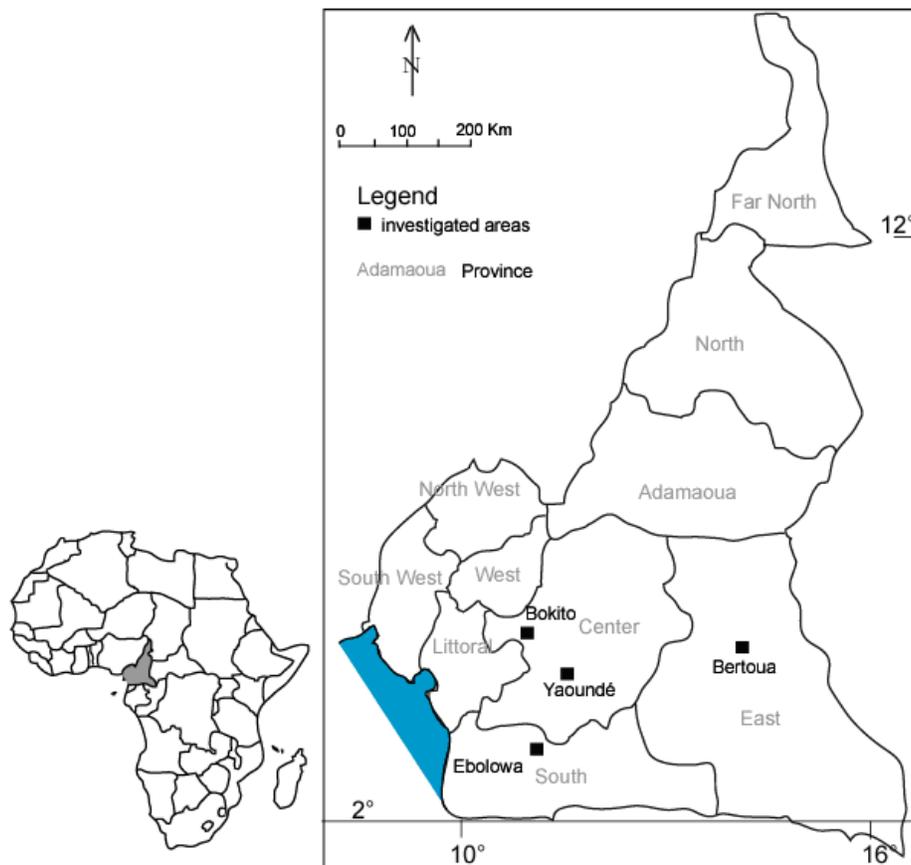
### Statistical analysis

Data were subjected to analysis of variance (ANOVA). Means were separated between treatments with the least significant difference (LSD), using the Statgraphic plus, version 5.0 (SIGMA PLUS) computer package. Comparisons were made among treatments of the same location site. A p-value < 0.05 was generally used to evaluate significance, although, higher levels were considered for p-value < 0.0001. The statistical package for social sciences (SPSS) was used to assess the correlation between the nodulation and growth parameters.

## RESULTS

### Differences in the chemical components of studied soils

The chemical properties of soils under study are shown in Table 1. The soil aluminium content was consistently lower in the fallow (0.34 meq/100 g) than in other LUSs at Bertoua ( $p = 0.025$ ), whereas at Bokito, it was



**Figure 1.** Localisation of Cameroon in Africa and distribution of the investigated sites in humid forest zones.

significantly lower ( $p = 0.013$ ) in the forest (0.28 meq/100 g) than the plantation (0.49 meq/100 g). At Ebolowa, the soil aluminium content was significantly lower in the fallow (0.32 meq/100 g;  $p = 0.002$ ). In contrast, no significant difference was found between the soil aluminium contents of different LUSs in Yaoundé. The lower aluminium values was 0.20 meq/100 g and the higher values was 0.52 meq/100 g.

Soil pH of the four sites of the humid forest zone ranged from 3.96 in the forest at Ebolowa to 6.00 in mixed farming at Bertoua and did not show significant differences between the LUSs in any of the studied sites. Ebolowa soils were more acid than those of Bertoua, Bokito and Yaoundé. Soil organic carbon ranged from 0.88 to 2.18%, while soil organic matter varied from 1.51 to 3.03%, with a mean value of 2.59%. At Bokito, the soil organic carbon was similar in forest, mixed farming and fallow, but was significantly higher ( $p = 0.017$ ) than in plantation. Similarly, the soil organic matter did not significantly vary between the Ebolowa mixed farming and forest, but was significantly higher ( $p = 0.05$ ) than those of the fallow and plantation. The total mean soil N value was low and ranged between 0.11 and 0.15% with little or no site variations. Plantation had the most acid LUS soil

with an average of 4.85.

### Nodulation of groundnut in different LUSs

All the soils used for nodulation experiment had indigenous rhizobia that were able to nodulate groundnut. The mean nodule number varies from 13 in the plantations to 94 nodules in the fallow, depending on site and LUSs. A highly significant correlation was found between nodule number and groundnut growth.

The number of nodules per plant was significantly higher ( $p < 0.0001$ ) in the fallow than in other LUSs in all the experimental sites, with the maximum number of nodules observed at Bokito (94 nodules). This nodulation status (>75 nodules/root system) is characterized as good according to Amijee and Giller (1998). There was no significant difference between the number of nodules formed in the plantation and forest at Bertoua, mixed farming, plantation and forest at Bokito and Yaoundé, mixed farming and plantation at Ebolowa. The number of nodules formed by *A. hypogaea* in all the LUSs at Ebolowa was lower than that of any of the sites, with the lowest accounting for plantation (13 nodules). This nodu-

**Table 1.** Variation of the soil chemical parameters under different land uses of the experimental sites in the humid forest zone of southern Cameroon.

| Site    | Chemical parameter           | Land use           |                    |                     |                    | p-value | LSD (5%) |
|---------|------------------------------|--------------------|--------------------|---------------------|--------------------|---------|----------|
|         |                              | Farm               | Fallow             | Plantation          | Forest             |         |          |
| Bertoua | Al <sup>3+</sup> (meq/100 g) | 0.47 <sup>b</sup>  | 0.34 <sup>a</sup>  | 0.40 <sup>ab</sup>  | 0.39 <sup>ab</sup> | 0.025   | 0.127*   |
|         | C/N (ratio)                  | 8.60 <sup>a</sup>  | 12.04 <sup>a</sup> | 9.40 <sup>a</sup>   | 8.65 <sup>a</sup>  | 0.561   | ns       |
|         | pH (H <sub>2</sub> O)        | 6.00 <sup>a</sup>  | 5.97 <sup>a</sup>  | 5.71 <sup>a</sup>   | 5.54 <sup>a</sup>  | 0.285   | ns       |
|         | N (%)                        | 0.12 <sup>a</sup>  | 0.11 <sup>a</sup>  | 0.11 <sup>a</sup>   | 0.11 <sup>a</sup>  | 0.846   | ns       |
|         | OM (%)                       | 1.77 <sup>a</sup>  | 2.05 <sup>a</sup>  | 1.78 <sup>a</sup>   | 1.60 <sup>a</sup>  | 0.549   | ns       |
|         | OC (%)                       | 1.03 <sup>a</sup>  | 1.20 <sup>a</sup>  | 1.03 <sup>a</sup>   | 0.93 <sup>a</sup>  | 0.535   | ns       |
| Bokito  | Al <sup>3+</sup> (meq/100 g) | 0.45 <sup>ab</sup> | 0.45 <sup>ab</sup> | 0.49 <sup>b</sup>   | 0.28 <sup>a</sup>  | 0.013   | 0.20**   |
|         | C/N (ratio)                  | 8.14 <sup>a</sup>  | 8.70 <sup>a</sup>  | 7.99 <sup>a</sup>   | 9.54 <sup>a</sup>  | 0.736   | ns       |
|         | pH (H <sub>2</sub> O)        | 5.68 <sup>a</sup>  | 5.71 <sup>a</sup>  | 5.15 <sup>a</sup>   | 5.68 <sup>a</sup>  | 0.830   | ns       |
|         | N (%)                        | 0.125 <sup>a</sup> | 0.112 <sup>a</sup> | 0.115 <sup>a</sup>  | 0.130 <sup>a</sup> | 0.450   | ns       |
|         | OM (%)                       | 1.72 <sup>ab</sup> | 1.67 <sup>ab</sup> | 1.51 <sup>a</sup>   | 2.12 <sup>b</sup>  | 0.097   | 0.61*    |
|         | OC (%)                       | 1.00 <sup>ab</sup> | 0.97 <sup>ab</sup> | 0.88 <sup>a</sup>   | 1.22 <sup>b</sup>  | 0.017   | 0.34**   |
| Ebolowa | Al <sup>3+</sup> (meq/100 g) | 0.52 <sup>c</sup>  | 0.32 <sup>a</sup>  | 0.37 <sup>ab</sup>  | 0.44 <sup>bc</sup> | 0.002   | 0.122**  |
|         | C/N(ratio)                   | 11.62 <sup>a</sup> | 18.89 <sup>b</sup> | 15.37 <sup>ab</sup> | 17.92 <sup>b</sup> | 0.041   | 6.29*    |
|         | pH(H <sub>2</sub> O)         | 4.09 <sup>a</sup>  | 4.02 <sup>a</sup>  | 4.14 <sup>a</sup>   | 3.96 <sup>a</sup>  | 0.783   | ns       |
|         | N (%)                        | 0.14 <sup>c</sup>  | 0.11 <sup>a</sup>  | 0.13 <sup>bc</sup>  | 0.12 <sup>ab</sup> | 0.005   | 0.01**   |
|         | OM (%)                       | 2.93 <sup>bc</sup> | 2.15 <sup>a</sup>  | 2.23 <sup>ab</sup>  | 3.03 <sup>c</sup>  | 0.05    | 0.77*    |
|         | OC (%)                       | 1.68 <sup>a</sup>  | 2.15 <sup>a</sup>  | 2.02 <sup>a</sup>   | 2.18 <sup>a</sup>  | 0.222   | ns       |
| Yaoundé | Al <sup>3+</sup> (meq/100 g) | 0.21 <sup>a</sup>  | 0.20 <sup>a</sup>  | 0.25 <sup>a</sup>   | 0.20 <sup>a</sup>  | 0.488   | ns       |
|         | C/N (ratio)                  | 12.14 <sup>a</sup> | 8.94 <sup>b</sup>  | 8.93 <sup>b</sup>   | 8.52 <sup>b</sup>  | 0.081   | 3.20*    |
|         | pH (H <sub>2</sub> O)        | 4.75 <sup>a</sup>  | 5.15 <sup>a</sup>  | 4.39 <sup>a</sup>   | 5.26 <sup>a</sup>  | 0.325   | ns       |
|         | N (%)                        | 0.125 <sup>a</sup> | 0.127 <sup>a</sup> | 0.145 <sup>a</sup>  | 0.135 <sup>a</sup> | 0.369   | ns       |
|         | OM (%)                       | 2.55 <sup>a</sup>  | 1.95 <sup>a</sup>  | 2.24 <sup>a</sup>   | 2.04 <sup>a</sup>  | 0.468   | ns       |
|         | OC (%)                       | 1.48 <sup>a</sup>  | 1.13 <sup>a</sup>  | 1.30                | 1.17 <sup>a</sup>  | 0.463   | ns       |

Values in a row followed by the same letter within a studied site are not significantly different at 5% level (ns: non-significant; \*, significant; \*\*, highly significant).

lation status (25 nodules/root system) was characterized as poor according to Amijee and Giller (1998). Poor nodulation occurs only in plantation and forest, while moderate nodulation (25 to 75 nodules/root system) were obtained in fallow and mixed farming. The number of nodules per plant was higher at Bokito and the lowest one at Ebolowa.

The dry weight of nodules harvested from plants at Bokito was significantly higher than that of any other studied site (Figure 2). Although, there were some differences in the nodules dry weight between LUSs, which was not statistically significant between the sites.

### Growth of *A. hypogaea* in different LUSs

The highest shoot dry weights of *A. hypogaea* were observed in Bokito soils, followed by Bertoua soils; whereas the shoot dry weights of plants in plantations were lower than those of other LUSs (Table 2). Fallow

significantly improved the fresh and dry weight of root and shoot systems of groundnut in Bertoua ( $p < 0.0006$ ), Bokito ( $p < 0.0009$ ) and Ebolowa ( $p < 0.007$ ) compared with mixed farming, plantation and forest. In Yaoundé in contrast, whereas the fresh weight of shoot system was significantly greater in the forest ( $p < 0.0001$ ) than in other land uses, the fresh weight of the root system was similar in mixed farming and fallow, but was at the same time significantly enhanced ( $p < 0.0001$ ) than in plantation and forest.

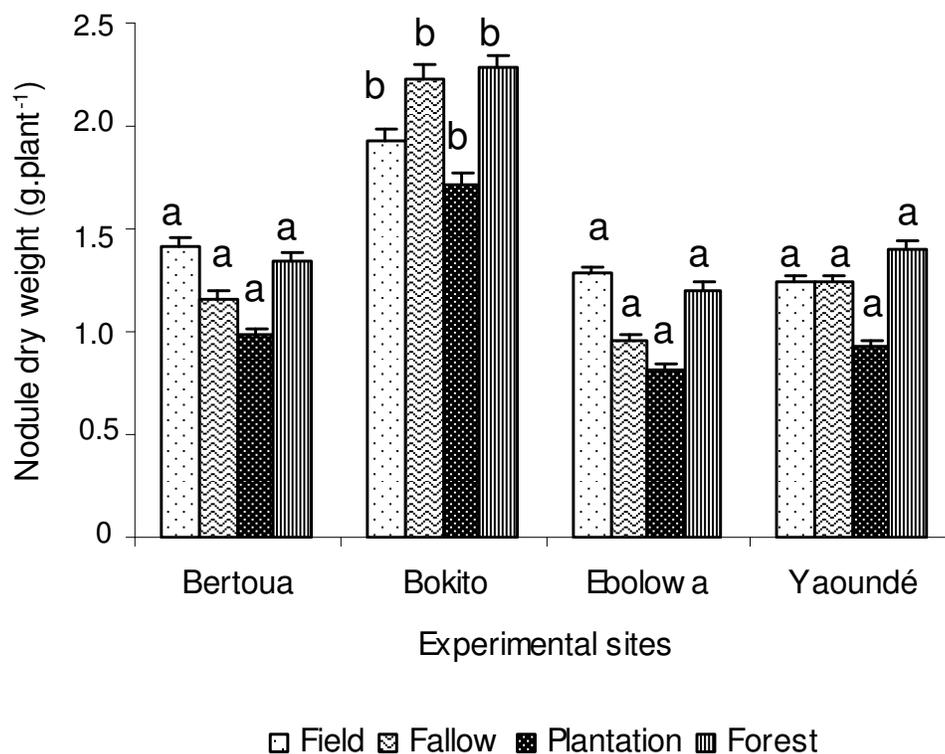
There was no significant difference ( $p = 0.321$ ) between the land uses as far as the dry weight of root system is concerned. The greatest plant fresh weight system was registered in a forest of Yaoundé (11.16 g), while the smallest was observed in a plantation of Ebolowa (4.03 g). The dry weight of the root system was generally not high, ranging from 0.11 g in plantations (Bokito) to 0.32 g in mixed farming (Yaoundé).

At Bokito, highly significant correlations were found

**Table 2.** Effect of LUSs on the biomass of *A. hypogaea* in four sites in humid forest zone of southern Cameroon.

| Study site | Parameter | Land use           |                    |                    |                    | p-value | LSD 5% |
|------------|-----------|--------------------|--------------------|--------------------|--------------------|---------|--------|
|            |           | Farm               | Fallow             | Plantation         | Forest             |         |        |
| Bertoua    | Fwss      | 8.50 <sup>b</sup>  | 9.08 <sup>b</sup>  | 5.77 <sup>a</sup>  | 5.90 <sup>a</sup>  | <0.0001 | 2.60   |
|            | Fwrs      | 1.51 <sup>ab</sup> | 1.85 <sup>b</sup>  | 1.48 <sup>a</sup>  | 1.55 <sup>ab</sup> | 0.102   | 0.36   |
|            | Dwss      | 1.26 <sup>a</sup>  | 1.67 <sup>b</sup>  | 1.11 <sup>a</sup>  | 1.06 <sup>a</sup>  | 0.0006  | 0.40   |
|            | Dwrs      | 0.15 <sup>a</sup>  | 0.25 <sup>b</sup>  | 0.17 <sup>a</sup>  | 0.18 <sup>a</sup>  | 0.0002  | 0.06   |
| Bokito     | Fwss      | 6.62 <sup>a</sup>  | 10.38 <sup>b</sup> | 5.44 <sup>a</sup>  | 6.96 <sup>a</sup>  | 0.0009  | 3.41   |
|            | Fwrs      | 1.35 <sup>c</sup>  | 1.30 <sup>c</sup>  | 1.09 <sup>ab</sup> | 0.94 <sup>a</sup>  | 0.002   | 0.26   |
|            | Dwss      | 1.04 <sup>a</sup>  | 2.02 <sup>b</sup>  | 0.85 <sup>a</sup>  | 1.10 <sup>a</sup>  | <0.0001 | 0.91   |
|            | Dwrs      | 0.12 <sup>a</sup>  | 0.20 <sup>b</sup>  | 0.11 <sup>a</sup>  | 0.12 <sup>a</sup>  | <0.0001 | 0.07   |
| Ebolowa    | Fwss      | 5.76 <sup>a</sup>  | 7.67 <sup>b</sup>  | 4.03 <sup>a</sup>  | 4.68 <sup>a</sup>  | 0.0004  | 1.91   |
|            | Fwrs      | 1.17 <sup>c</sup>  | 1.05 <sup>ab</sup> | 0.90 <sup>a</sup>  | 1.31 <sup>c</sup>  | 0.004   | 0.26   |
|            | Dwss      | 0.86 <sup>a</sup>  | 1.50 <sup>b</sup>  | 0.70 <sup>a</sup>  | 0.76 <sup>a</sup>  | <0.0001 | 0.63   |
|            | Dwrs      | 0.11 <sup>a</sup>  | 0.15 <sup>b</sup>  | 0.11 <sup>a</sup>  | 0.15 <sup>b</sup>  | 0.007   | 0.03   |
| Yaoundé    | Fwss      | 4.90 <sup>a</sup>  | 6.14 <sup>a</sup>  | 6.63 <sup>a</sup>  | 11.16 <sup>b</sup> | <0.0001 | 4.53   |
|            | Fwrs      | 1.40 <sup>b</sup>  | 1.61 <sup>b</sup>  | 1.94 <sup>c</sup>  | 0.99 <sup>a</sup>  | <0.0001 | 0.33   |
|            | Dwss      | 1.18 <sup>a</sup>  | 2.06 <sup>b</sup>  | 0.96 <sup>a</sup>  | 1.23 <sup>a</sup>  | <0.0001 | 0.82   |
|            | Dwrs      | 0.32 <sup>a</sup>  | 0.21 <sup>a</sup>  | 0.22 <sup>a</sup>  | 0.23 <sup>a</sup>  | 0.321   | ns     |

Fw, Fresh weight (g/plant); Dw, dry weight (g/plant); ns, non significant; rs, root system; ss, shoot system. Values in a row followed by the same letter within a studied site are not significantly different at 5% level.



**Figure 2.** Nodule dry weight of *A. hypogaea* from different LUSs in various sites. Bars marked by the letter within an experimental site are not significantly different at 5% level.

**Table 3.** Correlations between number of nodules per plant and the shoot and root biomass of groundnut from humid forest soils in southern Cameroon.

| Experimental site | Shoot and root biomass            |                                   |                                   |                                   |
|-------------------|-----------------------------------|-----------------------------------|-----------------------------------|-----------------------------------|
|                   | Fwrs                              | Fwss                              | Dwrs                              | Dwss                              |
| Bertoua           | $r = 0.579^{***}$<br>$p < 0.0001$ | $r = 0.774^{***}$<br>$p < 0.0001$ | $r = 0.055^{ns}$<br>$p = 0.665$   | $r = 0.635^{***}$<br>$p < 0.0001$ |
| Bokito            | $r = 0.468^{***}$<br>$p < 0.0001$ | $r = 0.692^{***}$<br>$p < 0.0001$ | $r = 0.461^{***}$<br>$p < 0.0001$ | $r = 0.474^{***}$<br>$p < 0.0001$ |
| Ebolowa           | $r = 0.081^{ns}$<br>$p = 0.522$   | $r = 0.503^{***}$<br>$p < 0.0001$ | $r = -0.010^{ns}$<br>$p = 0.935$  | $r = 0.406^{***}$<br>$p < 0.001$  |
| Yaoundé           | $r = -0.133^{ns}$<br>$p = 0.296$  | $r = 0.698^{***}$<br>$p < 0.0001$ | $r = -0.069^{ns}$<br>$p = 0.589$  | $r = 0.558^{***}$<br>$p < 0.0001$ |

Fw, Fresh weight; Dw, dry weight; ns, non significant; rs, root system; ss, shoot system; \*\*\*, very highly significant.

between nodule number and respectively the fresh weight root system ( $r = 0.468$ ;  $p < 0.0001$ ) and the dry weight root system ( $r = 0.461$ ;  $p < 0.0001$ ). Other significant and positive correlations were observed between the number of nodules and the dry weight of shoot system ( $r = 0.474$ ;  $p < 0.0001$ ) (Table 3). In contrast, there was no significant correlation between the number of nodules and the dry weight of root system at Bertoua, between the number of nodules and the dry weight of root system at Ebolowa and between the number of nodules and the dry weight of root system at Yaoundé. The positive correlation observed between nodulation and dry weight shoot growth of groundnut suggests that the two parameters evolve together.

## DISCUSSION

The soils of all the four LUSs were acidic with high aluminium content in most sites except Yaoundé. Since these mixed farming and fallow soils generally have never received inorganic fertilizer, the low pH value is assigned to growing lands. Except in Yaoundé, LUSs significantly influence the soil aluminium. The lower aluminium values, 0.20 meq/100 g is from the fallow and the higher values, 0.52 meq/100 g is from the mixed farming. In Bertoua and Ebolowa, the soil aluminium content was significantly lower in the fallow when compared with other LUSs. The low aluminium content in fallow could be due to the residual effect of the wood ash from the slash and burn practice. Agoumé and Birang

(2009) also reported that, aluminium saturation was significantly affected by the LUSs with the lowest aluminium saturation obtained in soils of *C. odorata* fallows. They noted that, soils of fallows presented a higher fertility level when compared with those of the secondary forests and cocoa plantations.

The different LUSs did not significantly influence soil acidity in all the experimental sites. Soils under investigation have pH values ranging from 3.96 to 6.00. Ebolowa soil was the most acidic of all (pH 4.07), in agreement with other results from which the soils of the southern region of Cameroon were acid with pH ranging from 3.69 to 4.12 (Fankem et al., 2006).

All the groundnut plants examined formed effective root nodules, suggesting that all these soils contain indigenous LNB able to nodulate *A. hypogaea*. The cross section of some nodules showed the red coloration, suggesting the presence of leghemoglobin, related to nitrogen efficiency (Abdel-Wahab et al., 2002). In all the experimental soils, no rhizobia inoculant strains were used. Nodules were thus, formed by indigenous LNB. These results are in accordance with other studies that showed that, nodulation failure is rare for many tropical legumes such as *A. hypogaea*, a promiscuous species belonging to cowpea miscellany group (Alwi et al., 1989).

Moderate to higher number of nodules, from 30 to 94 per plant was formed in the mixed farming and fallows in soils from humid forest zone in southern Cameroon. But can we consider that, natural nodulation in these LUSs is adequate? Assessments of more mixed farming nodulation and inoculation experiments are needed to

confirm this. Some results reported that, nodule number from indigenous rhizobia was higher in farm previously planted with groundnut than in other farms where groundnut was not previously planted (Bogino et al., 2006). The density of LNB in these soils can be compared with nodulation response of groundnut in the different LUSs to explain the results. The comparison supports the observation that, when LNB density in these LUSs is high, the nodulation is also high. LNB density is generally lower in cocoa or coffee plantations and forest soils than in fallows or mixed farming of southern Cameroon (Ngo Nkot, 2009). This could be the consequence of farming practices management involving pesticides in plantations and a legume for LNB in mixed farming and fallows. Thakuria et al. (2009) also observed a gradual increase in nitrogenase activities in roots of rice and pea under *Azospirillum/Rhizobium* alone integrated nutrient management (INM) or *Azospirillum/Rhizobium* plus phosphate-solubilizing bacteria dual INM plots in acidic soils, which could be a result of either population build-up of introduced bio-inoculants in the rhizosphere or better soil environment for the N fixers. The highest level of rhizobia genetic diversity was recorded in soils under cocoa plantation when compared with soils under mixed farming with groundnut (Ngo Nkot et al., 2008).

Soils from cocoa or coffee plantations and forests in southern Cameroon had the lowest number of groundnuts nodules, respectively 32 and 42 per plant. This suggests that, LNB were less abundant in these LUSs. The lack of response to inoculation has been previously demonstrated to be attributed to other factors such as inadequate nodulation, unfavourable conditions for survival of introduced LNB strains and inability of inoculant strains to compete with indigenous ones for nodule sites (Bogino et al., 2006). The mean number of nodules per LUS was very low in cocoa/coffee plantations, probably because these soils had been under cocoa or coffee plantations for the past 20 years at least, excluding legumes but regularly using fungicides and insecticides for disease and pest control. Ciani and Diriyé (1995) noted the absence of LNB in soils under banana and papaya plantations compared with soil were leguminous plants such as groundnut and cowpea were cultivated. Soils under plantations were the most acid of all the LUSs (pH 5.83 in average). By decreasing the LNB population density, acidity may delay the initiation of infection process and hence, the appearance of subsequent nodules. The decline of LNB in plantation may be the consequence of the lower soil pH. These findings are similar to other results reported by Cheng (2003), who showed that, *Medicago murex* grown in acid soil (pH 4.3) produced fewer nodules (2.3/plant) than plants grown at pH 7.0 (8.2/plant) 41 days after sowing. According to Brady and Weil (2002), the low pH in acid soils leads to an increased availability of aluminium and manganese which become toxic, as well as a low concentration of phosphate, calcium and molybdenum. The lack of adequate number of *Bradyrhizobium*

*japonicum* in Nigerian soils was shown to limit nodulation and nitrogen fixation of soybean, thus, decreasing the yield (Broomfiel and Ayanaba, 1980). The frequently low number of nodules observed in forest soils has been associated with a low requirement for N, which is considered not to be a limiting factor under these conditions (Zilli et al., 2004). Tematio et al. (2001) showed that, soils in the forest are not very fertile. The fertility is limited by soil acidity, the low amount of cations exchanged capacity, the aluminium toxicity and the low available phosphorus. In such conditions, LNB growth was reduced (Hussein, 2000; Zahran, 1999). Selection of acid tolerance rhizobial strains of *Phaseolus vulgaris* L. done by Gutierrez and Barraquio (2010) yielded that only 36 acid-tolerant strains over 189 (19%) were isolated in Philippines. Some local strains of rhizobia have been previously shown to be more acid tolerant than a Chinese one (Nwaga and Ngo Nkot, 1998). Further studies are needed to characterize, assess LNB density and select *A. hypogaea* strains adapted for acidic oxisols. According to Lindström et al. (2010) a challenge for agriculture is to match rhizobia and legume crops for optimal performance either by having plant genotypes adapted to local rhizobial populations or by inoculating effective strains adapted to prevailing environmental conditions and good competitive ability against local, less effective strains.

Nodules were present in large number at Bokito grey soils compared with Ebolowa reddish soil sites. This poor nodulation at Ebolowa clay soils might indicate its contribution for poor plant growth and low yield of *A. hypogaea*. Alemayehu (2010) also find poor nodulation of *Vicia faba* in Ethiopian soils and conclude that, poor BNF may partially be responsible for reduction of faba bean nodulation.

In Bokito sandy loam soils, the cropping systems are more diversified including *A. hypogaea* in the farms together with other legumes such as *Vigna unguiculata*, while in fallows, *Crotalaria juncea* was present. The improved soil fertility of Bokito site, due to the presence of more local LNB can account for the large density of nodules observed in this site. Remarkable increases in groundnut yield have been achieved through inoculation with *Bradyrhizobium* sp. mainly in areas cultivated with groundnut for the first time (Lanier et al., 2005).

Inoculation of groundnut by LNB provided contrasting responses on two sites in central region of Cameroon; since yield increases of 169% on a relatively fertile clayey loam soil from Yaoundé and of only 5% on a mixed farming low-fertility sandy loam soil from Bokito (Mandou et al., 2002; Nwaga et al., 2010). A synergistic effect of inoculation and molybdenum seed treatment was also noticed, since this dual treatment resulted in a 288% yield increase in the Yaoundé site and a 21% increase in the Bokito site, when compared with the untreated control. The contrasting response to inoculation in the two study sites may be related to the density of indigenous LNB populations, since the Bokito soil contained 17,000 cells/g and the Yaoundé soil only 170. Increase yield of

82% has been observed for *A. hypogaea* after rhizobia inoculation (Betsama, 1999). An inoculation response is extremely unlikely when native LNB soil bacteria density is more than 1,000 cells g<sup>-1</sup> (Thies et al., 1991; Mafongoya et al., 2004). Some preliminary results indicate that, soil infertility and low density of LNB could be one of the causes of low pod filling of groundnut.

The ability of *A. hypogaea* to nodulate in all the soils examined is an indication that the compatible LNB were present in these soils, but in diverse density. The growth of groundnut expressed in terms of shoot and root weight basis was better at Bokito and Bertoua than Yaoundé and Ebolowa soils, confirming the relation with nodulation results. Once again, growth was more improved in fallow than mixed farming, plantation and forest in all the experimental sites. The positive correlation observed between nodulation and dry weight, shoot growth of groundnut reflect that the LNB symbiosis enhance the plant response by providing increase nitrogen for the growth. Similar results were reported in sub Saharan Africa mixed farming inoculated with strains of LNB for cowpea (Ngakou et al., 2007), soybean (Megueni et al., 2006) and many other tropical legumes (Nwaga et al., 2010). Alemayehu (2010) also reported a positive correlation between nodulation and shoot height of *V. faba*.

According to Lindström et al. (2010) inoculation is recommended if the field has no history of legume cultivation, especially if the plant is exotic to a new environment or if the soil is acidic, saline or otherwise hostile to rhizobia. In this study, inoculation is more likely to provide significant increase in nodulation, nitrogen fixation and seed yield in plantations and forest where soils are acidic.

## Conclusion

Various soils of the humid forest zone of Cameroon contain LNB able to nodulate groundnut, but, are this enough for optimal nitrogen fixation and seeds production of *A. hypogaea*? Nodulation and growth of *A. hypogaea* closely depend on the land use system type and much more on the sites. An important variation was noticed on groundnut nodulation and growth according to site and land use. Further studies are needed to characterize major factors involved such as rhizobia density and select acidity tolerant strains adapted to environmental conditions of the humid forest zone of Cameroon. Investigations on these isolates could provide additional information on their symbiotic effectiveness with the aim of identifying very effective indigenous nitrogen fixing strains for local production of a specific groundnut inoculant.

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