Original Paper

Nitrogen Moderate Rates’ Effects on the Performance of Cocoa Seedlings (*Theobroma Cocoa* Linn.) in the Forest Zone of Togo (West Africa)

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Abstract
The cocoa trees nutrient requirements coverage, especially in nitrogen (N), remains essential for a better growth of the seedlings. The objective of this study was to determine the effects of the moderate nitrogen doses on the agronomic performance of the nursery cocoa seedlings in Togo’s forest zone. Trials were conducted in a complete randomized plot design with four replicates to identify the optimal complementary nitrogen dose helpful for better growth and nutrition of cocoa seedlings. The doses tested were 0; 0.5; 1 and 1.5 g.plant⁻¹ of urea (46% N). The results showed that the doses of 0.5 and 1 g.plant⁻¹ were the most likely to provide the best agronomic performance of nursery cocoa seedlings in the forest zone of Togo. However, the dose of 1 g.plant⁻¹ was optimal for good growth and balanced nutrition of cocoa seedlings and would therefore be recommended for the management of cocoa nurseries in the area.

Keywords
*theobroma cocoa, nursery, nitrogen, Togo*
1. Introduction

The cocoa tree (*Theobroma cacao* Linn.) is a cash crop with an increasing economic value (ICCO, 2016). Soils under cocoa are considered very rich for the nutrition of cocoa plant as the nitrogen content (N) was more than 3 g.kg\(^{-1}\), with an average between 1.26 and 2.25 g.kg\(^{-1}\) and a minimum of less than 0.5 g.kg\(^{-1}\). The organic matter content must be higher than 60 g.kg\(^{-1}\) and a C/N ratio of 25 indicated a very high soil fertility for cocoa production (Euroconsult, 1989; N’guessan et al., 2016). The soils in the cocoa production zone in Togo have a N content oscillating between 1 and 3 g.kg\(^{-1}\) (Koudjega & Tossah, 2009), hence a low to very good chemical fertility for cocoa trees. In principle, the soils in the Kloto area with a nitrogen content of 2 g.kg\(^{-1}\) and those of the Litimé area with a nitrogen content of 1 g.kg\(^{-1}\) will need N complement for good cocoa productivity. Paradoxically, the response of cocoa trees to nitrogen input is often mixed. In Nigeria, the works of Ojeniyi et al. (1982) showed that the nitrogen doses input of 0, 80, 160 and 240 kg.ha\(^{-1}\).ha\(^{-1}\) for 5 years in cocoa did not affect cocoa performance and yield. Most of the station tests results showed the importance of phosphorus and potassium in improving the cocoa yields. Calcium (Ca) and magnesium (Mg) inputs in small quantities in cocoa plants were also considered important (Snoeck, 2010). Nitrogen, which was often crucial in crop productivity, was absent in all the fertilizer formulae recommended for cocoa plantation in West Africa (Côte d’Ivoire: 0 N, 23 P\(_2\)O\(_5\), 19 K\(_2\)O, 10 CaO, 5 MgO; Ghana 0 N, 16 P\(_2\)O\(_5\), 20 K\(_2\)O, 10 CaO, 5 MgO). One wonders why the absence of N in the fertilizer formulae is recommended for cocoa? The answer to this question seems to come from the inherent needs of the plant itself. Exports of N soil according to Snoeck and Jardin (1992) by cocoa trees are 2.5 kg.ha\(^{-1}\) in nurseries (5-12 months of age), 140 kg.ha\(^{-1}\) for an immature orchard (28 months of age), 219 kg.ha\(^{-1}\) for an orchard in early production (39 months of age) and 453 kg.ha\(^{-1}\) for a mature orchard (50-87 months of age) while maize, for example, exports 100-180 kg.ha\(^{-1}\) of nitrogen in one production cycle (approximately 4 months) according to Adden et al. (2016). We are therefore facing a perennial crop that consumes less nitrogen than a cereal over the same growth period. The N requirements of cocoa therefore seem to be covered by nitrogen availability in most modern soils even though disparities may exist locally. Puentes-Paramo et al. (2014) have shown that nutrient input excess has a negative impact on cocoa productivity. Ribeiro et al. (2008) suggested a maximum dose of 240 mg of N per nursery pot; which corresponds to the dose of 0.52 g of urea per pot, for a better height and radial growth of the nursery cocoa plants. A better cocoa plant root growth is obtained with the maximum dose of 480 mg of N per pot or 104.3 mg of urea. In addition, Koudjega and Tossah (2009) demonstrated soil with N deficiency for cocoa trees in Togo’s agro-ecological forest zone. The lack of specific recommendations for the cocoa fertilizer in Togo was a constraint for a better productivity of this crop and requires the search for nutrient compensation of soils under cocoa, including nitrogen. The objective of this study was to determine the effects of moderate nitrogen doses on the agronomic performance of nursery cocoa seedlings in the forest zone of Togo.
2. Method

The nursery experiment was conducted for approximately six months in the “Centre de la Production de Matériel Végétal”/Plant Variety Production Center of Ezimé (CPMV) affiliated to Unité Technique Café-Cacao (UTCC, the Coffee Cocoa Extension Board in Togo). The center is located between the latitude 07° 29’31 “N and the longitude 0° 56’83” E with an altitude from 252 m to 4 km away from Amlamé along the National Road No 5 (Road network Atakpamé - Kpalimé) in the heart of Togo agro-ecological forest zone (Figure 1).

The experiment was carried out in a complete randomized plot design with four replications and a parameter which was the urea dose, to check spatial variability due mainly to the slope of the ground and the shading evolution according to the apparent movement of the sun. Each lot was made up of 25 pots separated from one another of 20 cm, a security interval required to avoid nutrient contamination between experimental units during watering and especially during the nitrogen input application. Two fresh cocoa beans were sown per pot. The thinning of the seedlings has been carried out three weeks after planting at a rate of a plant (the strongest) per pot.

The entire nursery was covered with translucent tarpaulin from the planting to the thinning to protect crops against the harmful effects of Harmattan. The watering was done regularly every two days between 5 pm and 6 pm based on the field capacity.

The black polyester pots used are those recommended for the research in Togo since the 1970s, the size...
of which are: 27 cm x 10 cm called “cocoa bag” (2120 cm³). The beans used are those of new varieties of cocoa (77 X 42 [N, 33%]; 77 X 85 [V, 34%]; 77 X 67 [R, 33%]) introduced in Togo in 2015 and originated from Ghana Cacao Board. The surface arable land (0-20 cm) was sampled in the center at the beginning of the tests, dried in ambient air, crushed and sieved manually to serve as a substrate in the pots. The sampled soil is clay-loamy, with a relatively low level of organic material (26 g kg⁻¹), poor in nitrogen (1.6 mg kg⁻¹) and phosphorus (18.4 mg kg⁻¹). The level of exchangeable bases was low (0.51 mg kg⁻¹ K; 9.20 mg kg⁻¹ Ca and 3.7 mg kg⁻¹ Mg) with a Cation Exchange Capacity (CEC) of 22 cmol kg⁻¹ and the pH was 6.7 (Adden & Kokou, 2016). The sampled soil has been correctly blended to ensure good homogeneity and used to fill every perforated pot while leaving some space for the irrigation water. The whole is placed under a shading of 4 x 3 x 2 m³ set to prevent the passage of 80% of the light (Mohd. Yusoff et al., 2007) from the beginning then calibrated for the conditioning five months later to allow 70% of the sun light.

An application of nitrogen alone without other major nutrients input in sufficient dose would seem to be a paradox compared to the laws of Liebig (1850) on the fertilization (law of the minimum). In fact, this trial was not looking for an optimal dose of N for cocoa seedlings but for the complementary dose of N existing in the soil to have the pre-established nutritional balance. The ratio between the main nutrients N, P, K, Ca and Mg has already been established by the work of Jardin and Snoeck (1992). The soil, used for the study being known deficient in N following these established reports, only the complement of N to be added is sought.

The treatments applied using Urea 46 % of N were as follows:

- **Treatment 1 (T1):** 0 g plant⁻¹ of urea;
- **Treatment 2 (T2):** 0.5 g plant⁻¹ of urea or 230 mg plant⁻¹ of fertilizing unit N;
- **Treatment 3 (T3):** 1 g plant⁻¹ of urea or 460 mg plant⁻¹ of fertilizing unit N;
- **Treatment 4 (T4):** 1.5 g plant⁻¹ of urea or 690 mg plant⁻¹ of fertilizing unit N.

These doses are established by moderating the doses already tested by Adden et al. (2015). The N doses were applied in a single input to the potted substrate three months after the sowing, after having diluted the urea in water; this to ensure that the seedlings have already exhausted the initial reserve of N. The other parameters remained the same for all the nursery seedlings.

The following growth variables were measured at the end of the test: the germination and emergence rates, length of the tap roots, stem girth, height of the seedling, number of leaves and foliar surface. The germination rate is calculated by dividing the number of beans germinated 15 days after the sowing by the number of sown beans multiplied by 100. The emergence rate is calculated by dividing the number of seedlings, emerged and alive one month after the sowing by the number of sown beans multiplied by 100. To measure the length of the root, three potted seedlings in each treatment were randomly selected and then tested. The tap roots were stripped and their length measured on graph paper. The length of a tap root in each treatment was the average of three measurements carried out on three tested seedlings. For the stem girth, the collar diameter of each seedling in a treatment was measured then the average
stem girth determined by multiplying the average diameter by π (3.142). The height of the seedlings is measured from the feet to the crown of the seedling. The foliar surface was calculated using the method of Bradfield (1962) referred to by Okon et al. (2013) with the formula:

\[ \text{Foliar surface of a leaf} = L \times l \times r \times n \]

Where: 
- \( L \) = Average length of the leaf
- \( l \) = average width of the leaf
- \( r \) = correction coefficient (0.72)
- \( n \) = number of leaves on the seedling

The Variance Analysis (Factorial ANOVA) with fixed effects and the Duncan Test, using the STATISTICA version 5.5 software (StatSoft Inc, 1999) at 5 % threshold, were performed. For the counting data (germination rate/emergence rate, number of leaves), the binomial distribution of the data was carried out by Poisson approach (Le Digabel, 2016).

3. Result and Discussion

The germination rate of cocoa beans averaged 98.25±2.91 % while the seedling emergence rate averaged 93.00±8.07 %. According to the different treatments, there was no statistical difference between the germination rates of beans two weeks after sowing (F(12); 16) = 1.52; p = 0.26) and the seedling emergence rates one month after sowing (F(12)16) = 0.24; p = 0.86). However, there was a significant difference between the germination rate of the beans and the seedling emergence rate (Figure 2). The distribution of the bean germination rate was normal around 98 % (Figure 3) while the distribution of the seedling emergence rate was normal around 93 % (Figure 4). The emergence of a certain number of beans could not be done correctly. This would be due to the severity of the effect of Harmattan during the test with very dry and cold winds that damaged a lot of cocoa and even coffee orchards during the 2015-2016 off-season in Togo. It should be noted that an average germination rate of 98 % and an average recovery rate of 93 % are quite significant in the extreme conditions of production.
The length of the tap roots of the cocoa seedlings averaged 19.8±4.23 cm (Table 1). The urea doses positively influenced the length of the tap roots (Table 2). The control (0 g.plant$^{-1}$) and the dose of 1.5 g.plant$^{-1}$ were found in the same statistical class (15.78±1.50 cm and 17.60±1.71 cm) then the doses of 0.5 and 1 g.plant$^{-1}$ generated the best lengths of tap roots (23.18±0.89 cm and 22.45±5.56 cm).

Figure 2. Cocoa Seeds Germination Rate vs Cocoa Seddlings Emergence Rate

Figure 3. Cocoa Seeds Germination Rate Distribution
There was a highly significant difference between the effects of different doses of urea on the length of the tap roots ($F(12)16)=5.70; p=0.01$). These results reflect the fact that the length of the roots is influenced by an input of a very moderate dose of urea of $1 \text{g.plant}^{-1}$. Such a dose probably influenced the phosphorus consumption positively (P) which would have a significant influence on the length of the root. The balanced ratio of N = 1.5P required in soils for a better growth of cocoa trees (Snoeck & Jardin, 1992) is probably reached with the maximum dose of $1 \text{g.plant}^{-1}$ of urea. This fact was justified the decrease in the length of the roots with the input of additional urea above $1 \text{g.plant}^{-1}$ of urea.

Moreover, the rate of the improvement in the root length under the effect of the input of N in relation to the control was 47 %, 42 % and 11 % respectively for the doses of 0.5, 1 and 1.5 g.plant$^{-1}$ of urea. The doses of 0.5 and 1 g.plant$^{-1}$ are more likely to improve the length of the cocoa seedlings roots.

**Table 1. Effects of Moderate Rates of Nitrogen Fertilizer on the Performance of Cocoa Seedlings**

| Nitrogen rate (46%N) | High, cm | Stem girth, cm | Leaves number | Foliar surface, cm$^2$ | Tap root length, cm |
|----------------------|---------|----------------|---------------|------------------------|---------------------|
|                      | Mean    | SD             | Mean          | SD                     | Mean               | SD        | Mean       | SD        |
| 0.0 g.plant$^{-1}$   | 33.75$^b$ | 2.63           | 3.93$^b$      | 0.41                   | 14$^b$             | 2         | 672$^a$    | 351       | 15.78$^b$ | 1.50      |
| 0.5 g.plant$^{-1}$   | 45.25$^a$ | 0.50           | 3.93$^b$      | 0.18                   | 17$^b$             | 1         | 1058$^a$   | 653       | 23.18$^a$ | 0.89      |
| 1.0 g.plant$^{-1}$   | 45.00$^b$ | 0.82           | 3.61$^a$      | 0.18                   | 17$^b$             | 2         | 1701$^a$   | 1275      | 22.45$^a$ | 5.56      |
| 1.5 g.plant$^{-1}$   | 41.75$^a$ | 8.42           | 2.98$^b$      | 0.18                   | 18$^b$             | 4         | 1135$^a$   | 697       | 17.60$^b$ | 1.71      |
| Means                | 43.88    | 6.87           | 3.61          | 0.46                   | 16$^b$             | 2         | 1141       | 822       | 19.8      | 4.23      |
| F                    | 5.85     | 11.87          | 1.44          | 0.39                   | 0.28               | 0.01*     | 0.00*      | 0.39      | 0.01*     |

* p ≤ 5 %; SD = Standard Deviation. The means in the same colon affected by the same letter were not significant with ANOVA and Test Duncan.
### Table 2. Analysis of Variance Structure of the Effects of Moderate Rate’s of Nitrogen Fertilizer on the Performance of Cocoa Seedlings

| Measured parameters         | Degree of freedom (DF) | Means square (MS) | DF error | MS error | F     | p-value |
|-----------------------------|------------------------|-------------------|----------|----------|-------|---------|
| High, cm                    | 3                      | 115.23            | 12       | 19.69    | 5.85  | 0.01*   |
| Stem girth, cm              | 3                      | 0.79              | 12       | 0.07     | 11.87 | 0.00*   |
| Leaves number               | 3                      | 8.23              | 12       | 5.73     | 1.44  | 0.28    |
| Foliar surface, cm²         | 3                      | 720315            | 12       | 664781   | 1.08  | 0.39    |
| Tap root length, cm         | 3                      | 52.59             | 12       | 9.22     | 570   | 0.01*   |

* p is significant at 5 % level.

The stem girth of the cocoa seedlings averaged 3.61±0.46 cm. Indeed, there was a significant disparity between the stem girth generated by the various treatments (Tables 1 and 2). The urea doses of 0; 0.5 and 1 g.plant⁻¹, gave stem girth of the same statistical class (respectively 3.93±0.41 cm; 3.93±0.18 cm and 3.61±0.18 cm) with significantly higher values (F(12)16 = 11.87; p = 0.00) at stem girth provided by the treatment 1.5 g.plant⁻¹ of urea (2.98±0.18). Clearly, by exceeding the dose of 1 g.plant⁻¹ of urea, the stem girth started decreasing whereas below 1 g.plant⁻¹ of urea, the input of nitrogen did not appear to be justified since the absolute control gave the same values, identical statistical quantities at the input of 0.5 and 1 g.plant⁻¹ of urea. This indeed confirmed that the high nitrogen doses can have inhibitory effects on cocoa seedlings according to Puentes-Paramo et al. (2014). The results of this study confirmed the work of Ribeiro et al. (2008), which gave cocoa seedling stem girth from 3.02 to 4.19 cm for cocoa seedlings fertilized at different doses of nitrogen (0 - 480 mg.pot⁻¹ of N).

The average height of cocoa seedlings was 41.44 ± 6.23 cm (Tables 1 and 2). This overall average has hidden diversity according to the various doses of urea applied. Two statistical classes were revealed according to the treatments (F(1216) = 5.85; p = 0.01). The absolute control (T1) remained less than an average height of 33.75±2.63 cm followed by all other treatments in the second higher class (45.25±0.50 cm; 45.00±0.82 cm and 41.75 ± 8.42 cm respectively for T2, T3 and T4 treatments), that was, an increase in seedlings height of 24 to 34 % compared to the control. Any nitrogen input induced an improvement in the height of the seedlings under the conditions of the experiment, justifying therefore, the need for additional nitrogen input to the nursery cocoa seedlings. The results of this study confirm those of the previous studies carried out by Souza-Junior and Carmello (2008); by studying the effect of nitrogen fertilization on the cocoa production, they found that the seedling height and stem diameter significantly responded to the input of nitrogen. Almeida et al. (2012) demonstrated the positive effect of nitrogen on the cocoa growth with a relative increase of 14 %.

The average number of leaves per cocoa seedling was 16 ± 2 leaves (Table 1). No statistical difference was observed between the various treatments (F(12)16) = 1.44; p = 0.28). The distribution of the
average number of leaves on plants is normal around 16 leaves (Figure 5) with a good clustering of Gauss bell-shaped variabilities. The foliar area on a cocoa tree seedling averaged 1141 ± 822 cm². No significant difference (Table 2) was noted between the foliar surfaces resulting from the different doses of urea applied (F(12).16) = 1.08; p = 0.39). The input of urea at the doses studied did not influence the number of leaves borne by the seedling or foliar area on the leaves of a seedling.

![NBRE_FEU; fit to Normal distribution: Kolmogorov-Smirnov d=,12313, p= n.s. Chi-Square: ,90166, df = 1, p = ,3423](image)

**Figure 5. Cocoa Seedlings Leaves Number Distribution**

The photosynthetic capacity of the cocoa seedlings would therefore remain the same regardless of the dose of urea applied. This assumes that nitrogen would be used more by other organs of the cocoa seedling than by the leaves. The height and radial growth as well as the root growth are more preferred at the young age of the cocoa seedlings.

This study tested the various nitrogen doses on the growth of cocoa seedlings. It allowed noting that the doses of 0.5 and 1 g.plant⁻¹ were the most likely to provide the best agronomic performance of nursery cocoa seedlings in the agro-ecological forests zone in Togo. Moreover, the dose of 1 g.plant⁻¹ was optimal for a good growth and a balanced nutrition of cocoa seedlings. It would be advisable to provide nursery cocoa seedlings with a dose of 1 g.plant⁻¹ of urea at the mid-point of the nursery’s duration. The dilution of the urea in water is very important to avoid the burns of cocoa seedlings.

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