

## **COBALT MEDIATED NITROGEN FIXATION BY DIFFERENT COWPEA CULTIVARS ENHANCED THE PERFORMANCE OF UPLAND NERICA7**

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### **ABSTRACT**

Nitrogen is an essential nutrient and the most limiting mineral, causing huge yield reductions in upland rice fields. Inorganic fertilisers are the major sources of nitrogen, however, their use threatens the environment owing to their contributions to greenhouse gases that trigger climate change. Biological Nitrogen Fixation (BNF) by leguminous crops such as cowpea is a sustainable, cheap and ecofriendly N source that is beneficial to field crops. The quantity and quality of total N fixation by diverse cowpea cultivars is sketchy. Nevertheless, the quantity of fixed N can be improved by cobalt-mediated nutrition, but the optimum rate needed by cowpea cultivars is unclear. This study investigated the performance of upland rice on sandy loam in response to the total N earlier fixed by different cowpea cultivars under different cobalt rates. The pot trial was carried out during early growing seasons of 2022 and 2023. In a 4 x 4 factorial combinations of four cobalt rates (0 (control), 0.04, 0.08 and 0.12 mg/kg) and four cowpea cultivars (SAMPEA11, Ife Brown, FUAMPEA2 and TVu-2882) were evaluated in a completely randomised design with six replicates. Ten kg pot, filled with sandy loam soil was used and a seed/pot each of the cultivars was sown. The cowpea cultivars were grown for 12 weeks after which they were harvested. Thereafter, upland NERICA7 was grown on the cobalt enriched soil previously grown with different cowpea cultivars. Growth and yield parameters of upland rice were collected at different growth stages. Also, the BNF efficiency was determined. Data were analysed using descriptive statistics, ANOVA and correlation at  $p < 0.05$ . SAMPEA11 on soil treated with 0.12 mg/kg cobalt fixed the highest N (25.81 g/kg) but this was not significantly higher than N fixed by TVu-2882 on soil enriched with the same cobalt rate. Growth indices of upland rice such as height, number of leaves and leaf area increased significantly on soil enriched with 0.08 mg/kg cobalt and grown with FUAMPEA2 but this was similar to the growth parameters recorded on soil treated with 0.12 mg/kg cobalt and grown with TVu-2882. However, yield and its components improved remarkably on soil treated with 0.12 mg/kg cobalt and grown with TVu-2882. TVu-2882 on soil treated with 0.08 mg/kg cobalt had the highest BNF efficiency relative to the other treatments. Grain yield correlated significantly with chlorophyll content ( $r=0.21$ ), biomass yield ( $r=0.62$ ) and harvest index ( $r=0.80$ ). Nitrogen fixation varied with different cowpea cultivars and was enhanced with cobalt enrichment. Variety TVu-2882 was highly efficient in fixing atmospheric N on sandy loam. Performance of upland NERICA7 improved tremendously on soil previously enriched with 0.08 mg/kg cobalt grown with TVu-2882.

**Keywords:** Nitrogen fixation efficiency, Cobalt mediation, TVu-2882 cultivar, Rice grain yield

## INTRODUCTION

Nitrogen fixing bacteria are able to situate in plant tissue as endophytes or closely associated with the roots of plants, including economically important crops. Their action in nitrogen fixation provides crops with nitrogen, an essential macronutrient (Hu, Wei, Ling, and Chen, 2021). Cobalt, a beneficial mineral, plays an important role in nitrogen fixation occurring within the nodules of legumes. Limited information on effects of Co on plant growth and crop yields necessitates further research to verify its essentiality in enhancing nodulation in leguminous crops (Vaseer *et al.*, 2020). Many studies have shown the importance of cobalt in facilitating atmospheric nitrogen fixation in leguminous crops (Gad, El-Moez, Bekbayeva, Karabayeva, and Surif, 2013; Minz *et al.*, 2018). However, little research attention has been channelled at understanding the efficiency of the fixed nitrogen in improving growth and yield of subsequent crops. It is one side for nitrogen to be fixed, it is another for the N fixed to be well mineralised and available for uptake by the crop in a rotation or relay cropping system.

Nitrogen Use Efficiency (NUE) is an integration of Nitrogen Uptake Efficiency (NUpE) governed by genetic and environmental factors. For cereals, NUE is often in the range of 10-25 kg grain per kilogram of N applied (Xu, Fan, and Miller, 2012). Information on NUE of subsequent crop grown after biological nitrogen fixation by a leguminous crop on sandy loam, is scanty.

Rice (*Oryza sativa* L.) is a major staple food globally and consumed across all socioeconomic classes. Rice consumption is increasing rapidly in Nigeria because of the shift in consumer preference, increasing population, increased income levels, and rapid urbanisation (Kamai, Omoigui, Kamara, and Ekeleme, 2020). According to Gu and Yang (2022), nitrogen is the most

limiting nutrient in agro ecosystem and it is required in large quantities for rice production. Despite the significant contributions of synthetic fertilisers, nitrogen requirement for rice production increases from year to year (Zhao *et al.*, 2022).

Besides, overuse of mineral fertilisers to meet up with the nitrogen requirement compromises soil health and agricultural sustainability (Can *et al.*, 2021). A possible alternative to overcome this problem could be through the development of Biological Nitrogen Fixation (BNF) mechanism in the rice farming system. Utilisation of BNF technology therefore, may decrease the use of fossil-based N fertiliser and reduce its harmful environmental effects to a considerable extent (Yang *et al.*, 2021). The BNF is viewed as a cheaper N than fossil-based fertilisers in improving soil fertility.

The need to assess the effectiveness of nitrogen fixation by different cowpea cultivars is germane to selecting the most appropriate cultivar(s) that would fix abundant and less volatile N for use by the subsequent crop in legume-cereal cropping system. Further, the need to ascertain the availability and extent of utilisation of the fixed nitrogen by the subsequent crop is pivotal to reducing inorganic fertiliser use in the rice farming system. It is also important to investigate how nitrogen fixed by different cowpea cultivars grown on cobalt enriched soil would improve growth and yield of subsequent cereal crops. It is premised on this that the performance of upland rice in response to nitrogen fixed by different cowpea cultivars under cobalt nutrition on sandy loam soil was investigated. The main objective of this study was to examine the performance of upland rice in response to Co mediated nitrogen fixation by four cowpea cultivars on sandy loam soil in Ibadan, southwestern Nigeria.

## MATERIALS AND METHODS

### **Location of the experimental site**

The pot trial was conducted at the Crop Garden of the Department of Crop Protection and Environmental Biology, University of Ibadan, Ibadan, Nigeria. The average rainfall during the study was 1230 mm, while the average temperature and relative humidity were 26.46°C and 74.5%, respectively.

### **Treatments and experimental design**

The soil pH and nitrogen concentration before the trial were 6.7 and 2.13 g/kg, as determined with a pH meter and micro Kjeldahl method, respectively. The treatments included four cobalt rates (0 (control), 0.04, 0.08 and 0.12 mg/kg cobalt) and four cowpea cultivars (SAMPEA11, Ife Brown, FUAMPEA2 and TVu-2882). The 16 treatments were arranged in a completely randomised design and replicated six times. The cowpea cultivars were grown for three months on sandy loam soil enriched with different rates of Co. After the cowpea was harvested, upland NERICA7 was sown on the same soil to assess the residual effect of the nitrogen fixed on the performance of upland rice. Rice seeds were first raised in a nursery, and transplanted at 14 days after sowing. Two weeks after transplanting, the seedlings were thinned, leaving one vigorous seedling per pot.

### **Data collection**

The following growth parameters were collected fortnightly per rice plant at vegetative, reproductive and physiological maturity stages: plant height (cm), number of leaves, leaf area (cm<sup>2</sup>), stem diameter (cm), chlorophyll content and the flag leaf inclination angle. The yield collected at physiological maturity stage included: number of panicles, number of spikelets, weight of 100 seeds (g), number of grains per plant (g) and grain yield (g/kg). The weights would be determined using a sensitive weighing balance.

Plant height was measured with the aid of a measuring rule in centimetres. The height was determined as the distance from the ground level to the tip of the youngest leaf. The number of leaves was obtained by counting the total number of fully formed leaves per plant. Leaf area was obtained by measuring the length and width of each leaf and leaf area was then computed based on the method described by Gomez (1972) as leaf area =  $K \times L \times W$ .

Where K= the adjustment factor (0.75); W= leaf width; L=leaf length.

The stem diameter was measured using a digital vernier caliper (Mitutoyo LCD Tube Thickness Caliper Series 573). The number of tillers was obtained by counting the number of tillers or auxiliary buds differentiated at the leaf axils. The number of panicles was determined by counting the number of panicles that emerged from the flag leaf sheath per plant.

### **Determination of angle of inclination and chlorophyll content**

The inclination angle between the culm and the flag leaf was determined by measuring the angle formed between the primary culm and the flag leaf using an inclinometer (Model 1080). The instrument was placed at a right angle to the primary culm. The angle formed between the culm and the flag leaf was recorded as the inclination angle according to the method of Yamashita et al. (2022). The chlorophyll content was measured using a chlorophyll metre (SPAD 502 Plus metre).

### **Determination of dry matter distribution**

The plant was harvested and oven dried at 80°C for 48 hours to ensure constant weight. The shoot and root of each plant were weighed and summed to obtain biomass distribution; this was expressed in g/plant.

**Determination of efficiency of nitrogen fixed by different cowpea cultivars as influenced by cobalt enrichment**

Efficiency of nitrogen fixed by each cowpea cultivar was determined as:  $BNF = \frac{A-B}{C}$  (Liu, Wu, Baddeley, and Watson, 2011). Where: BNF = Biological Nitrogen Fixation, A = Yield of cobalt mediated treatment (kg/ha), B = Yield of Non-cobalt mediated treatment (kg/ha) and C = Amount of Nitrogen fixed (mg/kg).

**Statistical analysis**

Data were analysed using descriptive statistics, Pearson Product Moment Correlation and analysis of variance

(ANOVA) with Statistical Analysis System (SAS Institute, 2002). The difference in means were separated using Least Significant Difference (LSD) at  $p \leq 0.05$ .

**RESULTS**

**Pre-trial soil analysis**

SAMPEA11 fixed the highest total nitrogen (25.81 g.kg<sup>-1</sup>) on soil amended with 0.12 mg/kg, while the lowest total N (16.04 g kg<sup>-1</sup>) was fixed by SAMPEA 11 on unenriched soil. The results revealed that nitrogen fixation by each of the cultivars evaluated was lowest in soil that was not augmented with cobalt (Table 1).

**Table 1: Concentration of N fixed by different cowpea cultivars on sandy loam enriched with cobalt**

Treatments	Total nitrogen (g/kg)
SAMPEA11+0.00 mg/kg cobalt	16.04p
Ife Brown+0.00 mg/kg cobalt	16.89o
FUAMPEA 2+0.00 mg/kg cobalt	18.03n
TVu-2882+0.0 mg/kg cobalt	19.64m
SAMPEA11+0.04 mg/kg cobalt	20.79j
Ife Brown+0.04 mg/kg cobalt	19.92i
FUAMPEA 2+0.04 mg/kg cobalt	20.26k
TVu-2882+0.04 mg/kg cobalt	22.25f
SAMPEA11+0.08 mg/kg cobalt	23.37cd
Ife Brown+0.08 mg/kg cobalt	21.40i
FUAMPEA 2+0.08 mg/kg cobalt	21.59d
TVu-2882+0.08 mg/kg cobalt	23.42c
SAMPEA11+0.12 mg/kg cobalt	25.81a
Ife Brown+0.12 mg/kg cobalt	21.79g
FUAMPEA 2+0.12 mg/kg cobalt	22.81e
TVu-2882+0.12 mg/kg cobalt	25.40ab

The values with same letter along the same column are not significantly different using Duncan’s Multiple Range Test at  $p > 0.05$  NH<sub>4</sub>N= ammonium nitrate, NO<sub>2</sub>=nitrate, NO<sub>3</sub>= nitrite, TN= total nitrogen.

**Height of upland NERICA7 in response to cowpea cultivars on soil treated with different cobalt rates**

Height of upland NERICA7 increased significantly on soil previously treated with different levels of cobalt and cowpea. At vegetative and tillering stages, NERICA7 was significantly taller (8.62 cm) on soil previously grown with SAMPEA11 and

treated with 0.12 mg/kg cobalt compared to the height of the plant recorded on unamended soil previously grown with TVu-2882. At stem elongation and panicle initiation stages, upland rice was tallest (20.85 and 26.05 cm) on soil previously treated with 0.08 mg/kg of cobalt and grown with FUAMPEA2. This was significantly taller than the height of rice plant (15.92 cm and 19.12 cm) on untreated soil sown with Ife Brown (Table 2).

**Table 2: Effect of cobalt mediated nitrogen fixation by different cowpea cultivars on height (cm) of upland NERICA7**

Treatments	Vegetative	Tillering	Stem elongation	Panicle initiation
SAMPEA11+0.00 mg/kg Co	7.00	14.92	17.87	22.28
SAMPEA11+0.04 mg/kg Co	7.17	13.65	17.68	21.12
SAMPEA11+0.08 mg/kg Co	7.40	12.72	16.90	22.55
SAMPEA11+0.12 mg/kg Co	8.62	15.77	19.08	22.88
Ife Brown+0.00 mg/kg Co	5.43	12.00	15.92	19.12
Ife Brown+0.04 mg/kg Co	6.87	13.23	16.82	21.00
Ife Brown+0.08 mg/kg Co	7.23	14.20	18.77	22.58
Ife Brown+0.12 mg/kg Co	7.55	12.78	19.13	21.58
FUAMPEA 2+0.00 mg/kg Co	5.78	13.08	16.98	22.43
FUAMPEA 2+0.04 mg/kg Co	7.05	15.03	19.00	23.12
FUAMPEA 2+0.08 mg/kg Co	7.13	15.53	20.85	26.05
FUAMPEA 2+0.12 mg/kg Co	8.27	15.03	18.85	23.32
TVu-2882+0.0 mg/kg Co	6.68	12.58	17.40	22.02
TVu-2882+0.04 mg/kg Co	7.32	14.12	18.37	23.32
TVu-2882+0.08 mg/kg Co	6.98	14.47	19.68	23.98
TVu-2882+0.12 mg/kg Co	7.56	14.37	19.45	25.23
LSD (p<0.05)	1.79	2.47	2.72	2.85

LSD = Least significant difference at  $p \leq 0.05$

**Number of leaves of upland NERICA7 in response to nitrogen fixed by cowpea cultivars treated with different cobalt rates**

NERICA7 formed more leaves on soil formerly treated with 0.12 mg/kg cobalt and grown with Ife Brown than the rice plant cultivated on unamended soil previously grown with SAMPEA11. However, this was not statistically higher than the number of leaves formed by NERICA7 on soil previously grown with FUAMPEA2 and treated with 0.12 mg/kg cobalt. At tillering, NERICA7 formed more

leaves (8.00) on soil previously grown with FUAMPEA2 and amended with 0.08 mg/kg cobalt, than leaves formed by the rice plant on unenriched soil grown with Ife Brown and TVu-2882 (Table 3). Upland NERICA7 formed more leaves (11.83 and 23.23) on soil amended with 0.04 mg/kg cobalt and previously grown with FUAMPEA2 at stem elongation and panicle initiation stages, respectively. This was significantly higher than number of leaves formed by upland rice on soil previously treated with 0.08 mg/kg cobalt and grown with SAMPEA 11 as well as on

unamended soil sown with Ife Brown, respectively, Table 3.

**Leaf area of upland NERICA7 in response to nitrogen fixed by cowpea cultivars treated with different cobalt rates**

NERICA7 formed wider leaf area (8.72 cm<sup>2</sup>) on soil previously grown with Ife Brown enriched with 0.12 mg/kg cobalt but this was not significantly higher than leaf area formed by NERICA7 other treatments (Table 4). At tillering, NERICA7 had wider leaf area (34.77 cm<sup>2</sup>) on soil earlier sown with SAMPEA11 and fertilised with 0.12 mg/kg cobalt compared to leaf area formed by NERICA7 on soil earlier grown with Ife Brown enriched with 0.12 mg/kg cobalt. At

stem elongation stage, NERICA7 formed wider leaf area (42.05 cm<sup>2</sup>) on soil earlier amended with 0.08 mg/kg cobalt and grown with TVu-2882 compared to leaf area formed by NERICA7 on unamended soil earlier grown with Ife Brown. This was not statistically higher than leaf area formed by NERICA7 grown on soil previously sown with SAMPEA11 and treated with 0.12 mg/kg cobalt. At panicle initiation, leaf area (52.57 cm<sup>2</sup>) formed by NERICA7 was wider on soil previously grown with FUAMPEA2 and amended with 0.12 mg/kg cobalt compared to leaf area formed by NERICA7 on soil previously amended with 0.08 mg/kg cobalt and grown with SAMPEA 11.

**Table 3: Effect of cobalt mediated nitrogen fixation by different cowpea cultivars on number of leaves of upland NERICA7**

Treatments	Vegetative	Tillering	Stem elongation	Panicle initiation
SAMPEA11+0.00 mg/kg Co	2.67	5.67	11.00	18.50
SAMPEA11+0.04 mg/kg Co	3.33	5.33	10.50	18.50
SAMPEA11+0.08 mg/kg Co	3.33	3.83	7.33	17.50
SAMPEA11+0.12 mg/kg Co	3.17	5.67	9.50	19.00
Ife Brown+0.00 mg/kg Co	3.00	4.33	8.83	15.83
Ife Brown+0.04 mg/kg Co	3.50	4.67	9.50	19.67
Ife Brown+0.08 mg/kg Co	3.50	5.67	10.83	22.50
Ife Brown+0.12 mg/kg Co	3.67	5.50	10.00	20.00
FUAMPEA 2+0.00 mg/kg Co	3.00	5.67	10.67	19.50
FUAMPEA 2+0.04 mg/kg Co	3.17	5.83	11.83	23.33
FUAMPEA 2+0.08 mg/kg Co	3.00	8.00	12.33	22.00
FUAMPEA 2+0.12 mg/kg Co	3.50	5.83	10.33	21.17
TVu-2882+0.0 mg/kg Co	2.83	4.33	8.67	18.00
TVu-2882+0.04 mg/kg Co	3.00	6.33	11.50	21.67
TVu-2882+0.08 mg/kg Co	3.00	5.33	11.17	22.17
TVu-2882+0.12 mg/kg Co	3.00	6.50	11.33	22.33
LSD (p<0.05)	0.67	2.53	3.38	3.46

LSD = Least significant difference at p ≤ 0.05

**Stem diameter of upland rice in response to nitrogen fixed by cowpea cultivars treated with cobalt rates**

Application of different rates of cobalt to soil cultivated with different cowpea cultivars had no significant effect on stem diameter of NERICA7. However, at stem

elongation, NERICA7 sown on soil augmented with 0.12 mg/kg cobalt and previously grown with FUAMPEA2 had thicker stem (3.64 mm) compared to stem diameter recorded in other treatments (Table 5).

**Table 4: Effect of cobalt on mediated nitrogen fixation by different cowpea cultivars on leaf area (cm<sup>2</sup>) upland NERICA 7**

Treatments	Vegetative	Tillering	Stem elongation	Panicle initiation
SAMPEA11+0.00 mg/kg Co	5.64	29.81	41.17	49.06
SAMPEA11+0.04 mg/kg Co	6.02	26.50	37.17	43.90
SAMPEA11+0.08 mg/kg Co	7.35	22.36	32.56	40.92
SAMPEA11+0.12 mg/kg Co	7.55	34.00	41.34	47.42
Ife Brown+0.00 mg/kg Co	6.20	21.04	32.31	41.24
Ife Brown+0.04 mg/kg Co	7.14	24.64	34.99	46.51
Ife Brown+0.08 mg/kg Co	7.26	28.33	37.89	46.68
Ife Brown+0.12 mg/kg Co	8.72	25.56	35.68	46.97
FUAMPEA 2+0.00 mg/kg Co	6.40	25.84	33.70	46.91
FUAMPEA 2+0.04 mg/kg Co	5.60	27.03	36.34	46.13
FUAMPEA 2+0.08 mg/kg Co	7.94	29.07	36.81	46.17
FUAMPEA 2+0.12 mg/kg Co	8.11	32.77	41.04	52.57
TVu-2882+0.0 mg/kg Co	6.19	26.56	38.38	46.65
TVu-2882+0.04 mg/kg Co	6.18	28.09	36.99	45.47
TVu-2882+0.08 mg/kg Co	5.94	26.67	42.05	50.50
TVu-2882+0.12 mg/kg Co	7.29	27.09	37.63	47.50
LSD (p<0.05)	18.12	7.46	7.34	8.53

LSD = Least significant difference at  $P \leq 0.05$

**Number of tillers of upland rice in response to nitrogen fixed by cowpea cultivars treated with different cobalt rates**

Number of tillers improved significantly at tillering stage on soil previously enriched with different rates of cobalt and different cowpea cultivars. At tillering stage, NERICA7 had more tillers (2.00) on soil treated with 0.04 mg/kg cobalt and previously grown with TVu-2882 compared to the number of tillers formed by NERICA7 on soil enriched with 0.08 mg/kg cobalt previously grown with SAMPEA11 or unenriched soil grown with TVu-2882 (Table 6). The treatments had no significant effect on tiller formation at stem elongation and panicle initiation stages.

**Flag leaf inclination angle and chlorophyll content of flag leaf in response to cowpea cultivars treated with cobalt rates**

Flag leaf of upland rice had higher inclination angle of 40° on soil enriched with 0.08 mg/kg cobalt and grown with TVu-2882 (Table 6). However, this was not significantly higher than the inclination angle of flag leaf of upland NERICA7 on soil enriched 0.04 mg/kg cobalt and sown with Ife Brown (34°).

Leaf of upland NERICA7 was greener (38.60) on soil treated with 0.12 mg/kg cobalt and grown with TVu-2882 than chlorophyll content recorded in upland rice grown on soil treated with 0.08 mg/kg cobalt and grown with SAMPEA 11 (Table 6).

**Table 5: Stem diameter (cm) of upland NERICA 7 in response to cowpea cultivars treated with different cobalt rates**

Treatments	Vegetative	Tillering	Stem elongation	Panicle initiation
SAMPEA11+0.00 mg/kg Co	5.64	0.22	0.23	0.24
SAMPEA11+0.04 mg/kg Co	6.02	0.22	0.23	0.24
SAMPEA11+0.08 mg/kg Co	7.35	0.23	0.24	0.25
SAMPEA11+0.12 mg/kg Co	7.55	0.21	0.22	0.27
Ife Brown+0.00 mg/kg Co	6.20	0.23	0.23	0.24
Ife Brown+0.04 mg/kg Co	7.14	0.21	0.22	0.23
Ife Brown+0.08 mg/kg Co	7.26	0.23	0.26	0.27
Ife Brown+0.12 mg/kg Co	8.72	0.24	0.25	0.26
FUAMPEA 2+0.00 mg/kg Co	6.40	0.23	0.24	0.25
FUAMPEA 2+0.04 mg/kg Co	5.60	0.23	0.25	0.26
FUAMPEA 2+0.08 mg/kg Co	7.94	0.23	0.24	0.26
FUAMPEA 2+0.12 mg/kg Co	8.11	0.23	3.64	0.24
TVu-2882+0.0 mg/kg Co	6.19	0.24	0.24	0.26
TVu-2882+0.04 mg/kg Co	6.18	0.20	0.22	0.24
TVu-2882+0.08 mg/kg Co	5.94	0.21	0.24	0.25
TVu-2882+0.12 mg/kg Co	7.29	0.23	0.25	0.26
LSD (p<0.05)	18.12	0.04	2.38	0.05

LSD = Least significant difference at  $P \leq 0.05$

**Yield of upland NERICA 7 in response to cowpea cultivars treated with cobalt rates**

Upland NERICA7 grown on soil previously enriched with 0.12 mg/kg and grown with TVu-2882 produced more spikelets (51.00) than the number of spikelets formed on soil previously sown with Ife Brown amended with 0.08 mg/kg cobalt. Also, on soil amended with 0.12 mg/kg and grown with TVu-2882, upland NERICA7 yielded more filled grains (392.00) relative to filled grains (245.00) formed by rice on soil previously grown with SAMPEA11 enriched with 0.08 mg/kg cobalt. However, this was not significantly higher than number of filled grain (391.00) formed by NERICA7 on soil previously amended with 0.04 mg/kg and grown with TVu-2882. NERICA7 sown in soil previously grown with Ife Brown treated with 0.12 mg/kg had the highest number of unfilled grains (50.00), which was not statistically different from number of unfilled grain (35.00) formed by NERICA7 sown in soil previously grown with

SAMPEA11 enriched with 0.08 mg/kg cobalt. NERICA7 had higher number of sterile panicles (18.90) on soil earlier grown with SAMPEA11 amended with 0.08 mg/kg cobalt. However, this was not significantly higher than number of sterile panicles observed in upland rice sown on soil previously amended with 0.12 mg/kg and grown with Ife Brown. Grain yield of upland rice was significantly higher (9.67 g/plant) on soil enriched with 0.04 mg/kg cobalt and sown with TVu-2882 than the grain yield recorded from rice grown on unenriched soil, previously sown with Ife Brown.

NERICA7 sown on soil previously grown with TVu-2882 and enriched with 0.04 mg/kg had higher biomass (22.10 g) than the biomass (16.99 g) accumulated by NERICA7 on unenriched soil previously grown with Ife Brown. The treatments had no significant effect on the harvest index of the upland rice.

TVu-2882 had highest nitrogen fixing efficiency on soil treated with 0.08 mg/kg

cobalt. This was statistically higher than nitrogen fixing efficiency of SAMPEA11

and TVu-2882 on soil treated with 0.08 mg/kg and 0.12 mg/kg cobalt, respectively.

**Table 6. Number of tillers, flag leaf angle and chlorophyll content of upland NERICA 7 in response to cowpea cultivars treated with different cobalt rates**

Treatments	Number of tillers			flag leaf inclination Angle (°)	Chlorophyll content
	Tillering	Stem elongation	Panicle initiation		
SAMPEA11+0.00 mg/kg Co	1.50	2.00	4.00	24	35.02
SAMPEA11+0.04 mg/kg Co	1.50	3.00	4.00	20	36.20
SAMPEA11+0.08 mg/kg Co	0.67	2.00	3.00	25	27.83
SAMPEA11+0.12 mg/kg Co	1.83	3.00	4.00	24	36.07
Ife Brown+0.00 mg/kg Co	1.17	2.00	3.00	33	26.87
Ife Brown+0.04 mg/kg Co	1.17	2.00	3.00	34	31.75
Ife Brown+0.08 mg/kg Co	1.67	2.00	4.00	22	32.77
Ife Brown+0.12 mg/kg Co	1.33	2.00	3.00	21	36.20
FUAMPEA 2+0.00 mg/kg Co	1.83	3.00	4.00	26	33.87
FUAMPEA 2+0.04 mg/kg Co	1.33	2.00	4.00	26	31.15
FUAMPEA 2+0.08 mg/kg Co	1.83	3.00	4.00	26	32.58
FUAMPEA 2+0.12 mg/kg Co	1.50	2.00	3.00	24	27.10
TVu-2882+0.0 mg/kg Co	1.00	2.00	3.00	22	28.52
TVu-2882+0.04 mg/kg Co	2.00	2.00	4.00	24	32.52
TVu-2882+0.08 mg/kg Co	1.50	3.00	3.00	40	31.63
TVu-2882+0.12 mg/kg Co	1.50	2.00	4.00	29	38.60
LSD (p<0.05)	0.83	0.90	1.40	13.3	9.88

LSD = Least significant difference at P ≤ 0.05

**Table 8: Effect of cobalt mediated nitrogen fixation by different cowpea cultivars on components of yield of upland NERICA7**

Treatments	Number of panicles	Number of spikelets	Number of filled grains	Number of unfilled grains	Sterile panicle (%)	Grain yield (g/plant)
SAMPEA11+0.00 mg/kg Co	3.00	38.00	307.00	2.00	1.50	7.94
SAMPEA11+0.04 mg/kg Co	3.00	38.00	306.00	2.00	3.10	7.84
SAMPEA11+0.08 mg/kg Co	3.00	37.00	245.00	35.00	18.90	6.67
SAMPEA11+0.12 mg/kg Co	3.00	37.00	275.00	1.00	0.60	7.57
Ife Brown+0.00 mg/kg Co	3.00	35.00	296.00	2.00	1.90	7.18
Ife Brown+0.04 mg/kg Co	3.00	37.00	288.00	3.00	3.00	7.52
Ife Brown+0.08 mg/kg Co	3.00	34.00	334.00	2.00	1.30	8.11
Ife Brown+0.12 mg/kg Co	3.00	40.00	260.00	50.00	17.50	7.07
FUAMPEA2+0.00 mg/kg Co	3.00	36.00	289.00	1.00	2.10	7.24
FUAMPEA2+0.04 mg/kg Co	3.00	39.00	300.00	3.00	4.00	7.67
FUAMPEA2+0.08 mg/kg Co	4.00	49.00	347.00	3.00	1.80	8.89
FUAMPEA2+0.12 mg/kg Co	3.00	40.00	291.00	2.00	1.50	8.03
TVu-2882+0.0 mg/kg Co	3.00	38.00	309.00	2.00	1.20	7.98
TVu-2882+0.04 mg/kg Co	4.00	49.00	391.00	2.00	1.00	9.67
TVu-2882+0.08 mg/kg Co	3.00	43.00	322.00	0.00	0.30	8.39
TVu-2882+0.12 mg/kg Co	4.00	51.00	392.00	3.00	2.40	8.78
LSD (p<0.05)	1.20	14.10	131.2	41.90	16.95	2.80

LSD = least significant difference at P ≤ 0.05

The length of panicle is highly significant and positively associated with other yield

components like number of spikelets, number of filled grain, weight of filled

grain, weight of 100 seeds, grain yield and harvest index. Weight of filled grain is significant and negatively correlated with number and weight of unfilled grain as well as with percentage of sterile grains. Number of unfilled grains had negative association with harvest index. The grain yield is significantly associated with other yield parameters such as length and number

of spikelets, number and weight of filled grains and weight of 100 seeds. Nevertheless, number of unfilled grains and percentage grain sterility are significant and negatively associated with grain yield. The chlorophyll content is significantly associated with weight of grains and grain yield. The inclination angle of flag leaf had significant association with harvest index.

**Table 9: Biomass, harvest index of upland rice and Biological nitrogen fixing efficiency of different cowpea cultivars mediated with cobalt nutrition**

Treatments	Biomass	Harvest index	BNF efficiency
SAMPEA11+0.00 mg/kg Co	18.94	0.44	0.01
SAMPEA11+0.04 mg/kg Co	20.29	0.38	0.44
SAMPEA11+0.08 mg/kg Co	17.83	0.36	-0.18
SAMPEA11+0.12 mg/kg Co	18.16	0.41	1.01
Ife Brown+0.00 mg/kg Co	16.99	0.42	0.01
Ife Brown+0.04 mg/kg Co	19.90	0.39	0.08
Ife Brown+0.08 mg/kg Co	18.00	0.45	0.85
Ife Brown+0.12 mg/kg Co	18.79	0.37	0.10
FUAMPEA2+0.00 mg/kg Co	18.68	0.38	0.02
FUAMPEA2+0.04 mg/kg Co	17.98	0.43	0.78
FUAMPEA2+0.08 mg/kg Co	18.87	0.46	0.85
FUAMPEA2+0.12 mg/kg Co	18.92	0.42	0.26
TVu-2882+0.0 mg/kg Co	20.53	0.38	0.02
TVu-2882+0.04 mg/kg Co	22.10	0.43	0.44
TVu-2882+0.08 mg/kg Co	18.02	0.46	1.50
TVu-2882+0.12 mg/kg Co	19.86	0.43	-0.22
LSD (p<0.05)	4.45	0.11	1.64

LSD = Least significant difference at  $p \leq 0.05$

**Table 10: Correlations of yield components and yield with chlorophyll and flag leaf angle of upland NERICA7 under biological nitrogen fixation with cowpea cultivars mediated with cobalt enrichment**

	LP	NS	NF	WF	NU	WU	STR	W100S	GY	BM	HI	CHL	AG
LP	1	0.292**	0.300**	0.321**	0.032	0.104	0.031	0.327**	0.338**	0.072	0.357**	-0.058	0.145
NS		1	0.824**	0.720**	-0.001	0.156	-0.013	0.159	0.750**	0.493**	0.535**	0.131	0.131
NF			1	0.914**	-0.346**	-0.115	-0.363**	0.336**	0.918**	0.561**	0.721**	0.149	0.092
WF				1	-0.406**	-0.217*	-0.441**	0.529**	0.994**	0.600**	0.808**	0.199*	0.108
NU					1	0.728**	0.969**	-0.533**	-0.332**	-0.074	-0.424**	0.056	-0.047
WU						1	0.816**	-0.369**	-0.109	0.065	-0.250*	0.020	-0.073
STR							1	-0.547**	-0.357**	-0.082	-0.455**	-0.001	-0.054
W100S								1	0.497**	0.131	0.590**	0.142	0.175
GY									1	0.618**	0.795**	0.205*	0.102
BM										1	0.040	0.129	-0.136
HI											1	0.181	0.242*
CHL												1	-0.105
AG													1

\* significant at  $p < 0.05$ , \*\* significant at  $p < 0.01$  LP = Length of spikelets NS= number of spikelets, NF = number of filled grain, WF = weight of filled grain, NU, number of unfilled grain, WU= weight of unfilled grain, STR = % Sterility, = W100S = weight of 100 seeds, GY = grain yield, BM = biomass, HI = harvest index, CHL = Chlorophyll content, AG= inclination angle

## DISCUSSION

It was apparent that enriching soil with cobalt could enhance the extent to which cowpea could fix atmospheric nitrogen. All the cowpea cultivars fixed substantial amount of nitrogen on cobalt-enriched soil than the N quantity fixed into soil where no cobalt was applied. (Vaseer *et al.*, 2020) has reported similar findings on mung bean. Response of different cultivars varied with rates of cobalt applied, with the highest N recorded at the maximum concentration assessed in this study. Apparently, SAMPEA11 that fixed highest concentration of N also fixed the lowest into unenriched soil. This suggests that the cultivar is likely to be a poor nitrogen fixer under natural conditions. On the other hand, TVu-2882 could be described as a good nitrogen fixer over and above other cultivars as it fixed about 77.3% nitrogen into unenriched soil compared to N fixed into soil treated with 0.12 mg/kg cobalt by this cultivar. The variation in the extent of N fixed by the different cowpea cultivars could be attributed to the variations in their genetic composition. Seido, Santos, Fernandes, da Silva, and Matos (2019) have shown that the BNF efficiency varies with cultivars. TVu-2882 cultivar would be a better option that could be recommended in rotation or relay cropping system with cereal crops owing to its remarkable nitrogen fixing abilities compared to other commonly grown cultivars. Our observation agrees with that of Simunji, Munyinda, Lungu, Mweetwa, and Phiri (2019) who reported that different cultivars had varying nitrogen fixing potentials in response to vagaries of edaphic factors.

Growth of upland rice improved tremendously on soil fixed with the highest nitrogen. Cowpea is a good soil N fixer, especially on soil low in nitrogen (Masete, Munjonji, Ayisi, and Mopape-Mabapa (2022)). It was obvious that cobalt enrichment facilitated BNF by some cowpea cultivars better than others. In this study, all the cowpea cultivars fixed

appreciable N quantity which was available for uptake by the subsequent crop. Nevertheless, SAMPEA11 fixed the most nitrogen, which improved the fertility of the soil N thereby enhancing growth and development of upland rice. Upland rice will benefit from nitrogen fixed by the cowpea in the cowpea-upland rice rotation system and this will go along with reducing the mineral fertiliser application for growing upland rice (Borges *et al.*, 2023). Upland rice requires a lot of nitrogen to produce substantial biomass necessary for partitioning adequate assimilate into grain. The quantity of N fixed by the cultivars evaluated in this trial was sufficient to sustain upland rice growth and yield. Kumar *et al.* (2020) have shown that under legume-cereal rotation, legumes can synthesise up to 300 kg N/ha per year.

Our study showed that SAMPEA11 fixed more than 500 kg N/ha per growing cycle on soil enriched with 0.12 mg cobalt. The amount of nitrogen fixed is higher than the quantity reported by Awonaike, Kumarasinghe, and Danso (1990) under natural conditions. The amount of N fixed by the cultivars ranged from 320 to 516 kg N/ha, which was far more than 240 kg N/ha required for growing upland rice. The fixed nitrogen is expected to have far-reaching effects on the performance of the subsequent crop cultivated on such soil. Since application of external nitrogen fertiliser may no longer be necessary, this will have a remarkable effect in increasing rice production, reduce production cost, increase growers' income and ensure food security. Masuda *et al.* (2023) have shown that paddy rice benefits greatly from BNF when in rotation with leguminous crops.

Angle of inclination has a strong relationship with capturing of solar rays and assimilate partitioning (Pisek, Řezníčková, Adamson, Ellsworth, and Williams, 2021). Inclination of flag leaf determines the positioning of the leaf for appropriate interception of precipitation and sunlight for photoassimilate production (Li, Fang,

and Zhang, 2023). The steeper the flag leaf inclination angle, the lesser the amount of light intercepted. This further expresses the carbon assimilatory and partitioning potentials of the flag leaf, which is linked to nitrogen availability and other complex pathways according to Song, Qi, Ahammed, Yu, and Xia (2022). It is most likely that a substantial volume of nitrogen uptake was traceable to the linearity of nitrogen fixed by diverse cowpea cultivars to the rate of cobalt applied. This could explain the reason why the upland rice grown on soil enriched with a higher amount of cobalt and grown with TVu-2882 showed a remarkably high inclination angle than on other treatments. Falster and Westoby (2003) have shown that leaf architecture varies across species as well as environmental influences.

The substantial increase in chlorophyll content of upland rice on soil treated with upper cobalt limit and grown with TVu-2882 may be ascribed to the amount of the fixed nitrogen fixed by this cultivar (Ali, Hayat, Hayat, and Ahmad, 2010). Biological nitrogen fixation is a function of the species involved, compatibility of the nitrogen fixing bacteria with the root of the host plant and suitable environmental conditions (Ladha *et al.*, 2022). Obviously, cobalt mediated nutrition could improve nitrogen fixation on sand loam low in nitrogen concentration by leguminous crop (Hu *et al.*, 2021).

The BNF mediated with cobalt nutrition enhanced yield and yield components of upland rice (Omwocha, 2020). Yield of upland was better on soil treated with cobalt than on the soil that were left unamended. It appeared as if TVu-2882 was a better cultivar for fixing nitrogen on sandy loam soil as revealed by the outcome of the yield components. Besides, the cultivar requires minimal cobalt to effect higher nitrogen fixation with greater yield indices than other cultivars. The yield indices showed that TVu-2882 could supply adequate nitrogen that could sustain reproductive

pathways without compromising the yield. The minimum sterile panicles and unfilled grain under TVu-2882 on cobalt enriched soil infers that it supplied sufficient nitrogen the high effectiveness in enhancing yield indices. Evidently, the improved yield components observed under this treatment translated to a better grain yield. Upland rice benefited from the previously fixed nitrogen on sandy soil to accumulate biomass and photoassimilate that were translocated into the sink as economic benefits for growers (Masuda *et al.*, 2023).

Generally, biomass accumulation varied with different rates of cobalt application as well as cowpea cultivars. Augmentation of sandy loam with the lowest cobalt rate improved nitrogen fixation as the N fixed at this rate increased biomass accumulation by upland rice in the order TVu-2882>SAMPEA11>Ife

Brown>FUAMPEA2. This suggests that the quantity of N fixed by the cultivars at this Co rate was sufficient to effect optimal biomass distribution into both morphological and economic yield.

Nitrogen fixing efficiency of cowpea intensified on soil enriched with 0.08 mg/kg cobalt on soil grown with TVu-2882. It is expected that upland rice benefited immensely from the N fixed under TVu-2882+0.08 mg/kg Co treatment than other treatments. Nitrogen, an indispensable macronutrient and component of nucleic acids as well as all amino acid plays irreplaceable roles in the growth of upland rice. Therefore, growing TVu-2882 on nitrogen limiting soil enriched with cobalt, a beneficial mineral that promotes oxidation of nitrogenase in cowpea nodule is very paramount (Jaiswal, Mohammed, Ibny, and Dakora, 2021).

## CONCLUSIONS

The level of nitrogen fixed on sandy loam by different cowpea cultivars varies. Also, enrichment of sandy loam with cobalt increased nodulation and nitrogen fixing

potentials of different cowpea cultivars. The quantity of nitrogen fixed by different cowpea cultivars increased with an increase in the concentration of cobalt. TVu-2882 was more efficient in fixing atmospheric nitrogen on soil enriched with 0.08 mg/kg cobalt. In this study, nitrogen fixed ranged from 16.04 g/kg by SAMPEA11 on unenriched soil to 25.81 g/kg by SAMPEA11 on sandy loam enriched with 0.12 g/kg. Growth, biomass and yield of upland rice NERICA7 improved remarkably on soil previously enriched with 0.08 g/kg and sown with TVu-2882. Enrichment of sandy loam grown with cobalt and sowing with TVu-2882 is recommended to enhance nitrogen fixation necessary for growing upland rice. Therefore, for a sustainable supply of nitrogen and increasing grain yield of upland rice, biological nitrogen fixation is recommended. This is important to increase nutrient recycling, reduce upland rice production cost and minimise harmful effect of inorganic nitrogen usage in rice agroecosystem.

#### **Author contribution statement**

Oyeyemi A. Dada (OAD) conceived the research idea, prepared the proposal, collected and analysed the data, and wrote the manuscript. Oluwaseun P. Akinsola, supervised by the OAD carried out the experimentation, data collection and management. Both authors contributed equally and approved the final manuscript.

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#### **Compliance with ethical standards**

The authors complied with the ethical standards required for conducting research as laid down by the University of Ibadan Codes of Ethics.

#### **Data Availability**

The set of data used in this study has not been published elsewhere either partly or in its entirety. The data sets used and/or analysed during the current study are available from the corresponding author on request.

#### **Conflict of Interest**

The authors declare that there are no competing interests.

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