

Original Research Article

**Response of Soybean [*Glycine max* (L.) Merrill]
to Local Nitrogen Fixing Bacteria Inoculation in
Divo Area, Center-south of Côte d'Ivoire**

UNDER PEER REVIEW

ABSTRACT

Experiment was conducted on farm in Divo to evaluate the elite native rhizobia strains on soybean nodulation, growth and yield. The experiment was layout in a randomized complete block design with three replications. The treatments include seven native strains, one exotic strain (*Bradyrhizobium japonicum* IRAT FA3), one synthetic fertilizer NPK 12-22-22 formulation and one negative control. Agronomic parameters (plant height, plant dry biomass, nodules number plant⁻¹, pods number plant⁻¹, pods weight plant⁻¹, 100-seeds weight and seeds yield ha⁻¹) were measured. Results showed that all tested local rhizobia enhanced nodules number, plant growth and soybean yield as compared to negative control. Among these native rhizobia, RSC324 and RSC502 induced more nodules than the introduced strain *B. japonicum* IRAT FA3. These same bacteria induced more pods per plant than the introduced bacterial strain IRAT FA3 and synthetic fertilizer (12-22-22). They induced the the highest pods number plant⁻¹ (144 and 133 pods plant⁻¹ respectively) and seeds yield (2.5 t.ha⁻¹). In addition to these bacteria, the local strain RSC508 increased soybean pods yield compared with synthetic fertilizer. Therefore, this study suggests that local rhizobia RSC324 and RSC502 proved to be the most effective and competitive strains for soybean cultivar Canarana and could be use for inoculum sources in Divo area.

Keywords: Local nitrogen fixing bacteria, inoculation, soybean, forest zone, Côte d'Ivoire

1. INTRODUCTION

Soybean (*Glycine max* L. Merrill) is a food legume prized for its exceptional richness in protein. It is also known for its ability to fix atmospheric nitrogen in symbiosis with *Bradyrhizobium* sp [1 ; 2]. This symbiotic fixation capacity is an economic and ecological potential for sustainably restoring the fertility of depleted soils. This crop is seen as a sustainable solution for small producers for soil management and an additional economic source in developing countries. In Côte d'Ivoire, soybean cultivation is mainly grown in the north and center of the country [3]. Actually, the scarcity of agricultural land in the Ivorian forest zone, where the availability of subsistence foodstuffs is becoming a major issue, the introduction of soybean into cropping systems deserves to be popularized. Indeed, the pedoclimatic conditions of this area, favorable to agriculture, have favored the establishment of perennial crops such as cocoa, coffee, rubber and oil palm [4]. The high propensity of farmers for these perennial crops has caused strong pressure on available agricultural resources. Divo department is one of the areas where the situation is becoming increasingly worrying. This department has lost 86 % of its forest area in less than half a century in favor to industrial crops. Forest cover fell from 569,182 hectares in 1960 to 79,680 hectares in 2020 [5]. As a result, land intended for subsistence farming has been used to expand cocoa, rubber and oil palm plantations [6]. This situation has led to strong pressure on the relics of agricultural land, resulting in their impoverishment [7]. Thus, to meet ever-increasing food needs, fallow land, which was the natural soil management method, is being abandoned in favour of a method dependent on synthetic fertilizers. However, the use of these chemical inputs has negative consequences for the environment, farmers' incomes and their well-being [8]. In this context, the use of soybean in cropping systems could make it possible to reduce the use of these chemical inputs, with the advantages offered by the soybean-nitrogen fixing bacteria symbiosis in this area of high population density and short fallow periods of soils. Successful introduction of soybean into the first time on the new areas is dependent on inoculation. The choice of bacteria used to produce the inoculum must take into account their efficiency, competitiveness and ability to adapt to local agro-ecological conditions to enable them to express their full potential [9]. In Côte d'Ivoire, studies carried out in Bouaké and Daloa have demonstrated that local rhizobia strains are efficient, competitive and adaptable to local environmental conditions [3 ; 10]. However, there is little information on the use of local rhizobia strains in Divo area. Therefore, this study was undertaken to evaluate the symbiotic and agronomic performance of different local rhizobia strains on soybean in Divo area.

2. MATERIAL AND METHODS

2.1 Description of experimental sites

The experiment was conducted on ferrallitic soil at Divo area during the cropping season 2019-2020. Field Trial was established at Divo (5°52'48,53"N ; 5°21'28,10"W). The physical and chemical characteristics of initial soils of this trial location are shown in Table 1. The soil at this site is predominantly sandy (57.64 %), with a high proportion of silt (26.9 %). The soil is slightly acidic, with a pH of 6, and contains high levels of organic matter (4.23 %), total nitrogen (0.19%) and available phosphorus (70 mg/kg). Trial was established in 2019, during the short rainy season between august and November. During this period, temperatures varied about 25 °C to 28 °C and rainfall distribution varied about 60 mm to 158 mm monthly. The previous crop at this location was corn (*Zea mays*). This site had no history with soybean cultivation or inoculation with nitrogen fixing bacteria.

Table 1 : Physical and chemical characteristics of the experimental site soil

Physical parameters			pH _{H2O}	OC (%)	Nt (%)	OM (%)	Pass (mg/kg)	Ca ²⁺	Mg ²⁺	K ⁺	Na ⁺	CEC
Clay (%)	Silt (%)	Sand y (%)										
14,5	26,9	57,64	6	2,46	0,19	4,23	70	2,309	1,049	0,243	0,077	10,80

OC : organic carbon; Nt : total Nitrogen ; OM : organic matter, Pass : available phosphorus; CEC : Cation exchange capacity.

2.2 Materials and inoculum production

The seeds of soybean [*Glycine max* (L.) Merrill] cultivar Canarana were obtained from the Crops Research Station (SRCV) of the National Center for Agronomic Research(CNRA) in Bouaké (Côte d'Ivoire). This soybean cultivar growth cycle is 120 days. Rhizobial strains used to inoculum formulation in this work consisted of seven (07) native rhizobia (RSC115, RSC119, RSC309, RSC324, RSC502, RSC504 and RSC508) isolated from soybean and one (01) exotic strain (*Bradyrhizobium japonicum* IRAT FA3) used as reference. Local and exotic rhizobia were provided respectively by the Laboratory of Agrovalorization of Jean Lorougnon Guède University in Daloa (Côte d'Ivoire) and the Central Laboratory of Soils, Water and Plants (LCSEP) of the CNRA in Bouake (Côte d'Ivoire). Each bacterial strain was grown in YEM liquid medium and packaged in solid form in previously treated and sterilized peat when the density of each pre-culture was estimated at 10⁹ CFU/mL.

2.3 Experimental design and treatments

Experiment was laid out in a randomized complete block design with ten treatments replicated three times. Treatments included seven native strains ((RSC115 (T1), RSC119 (T2), RSC309 (T3), RSC324 (T4), RSC502 (T5), RSC504 (T6) and RSC508 (T7)), one exotic strain (*Bradyrhizobium japonicum* IRAT FA3), one synthetic fertilizer NPK 12-22-22 formulation (TN) and one without treatment (T0). The experimental set-up comprised a total of 30 elementary plots. Each plot measured 6 m² (3 m x 2 m) with an alley of 1 m between plots. A plot was composed of seven sowing lines with 10 pockets per line. Consecutive blocks were separated of 2 m. In each plot, effective area was 3 m².

Before sowing, seeds were mixed thoroughly with peat-based inoculum. The seed coating inoculation method was used at 60 kg of seeds for a dose of 400 g of inoculum. Controls plots were sowed first in the experiment in order to avoid contamination with tested inoculum. The fertilized control received basal fertilizer at the rate of 150 kg.ha⁻¹. Three (3) seeds were sowing per pocket with a spacing of 50

cm between rows and 20 cm between pockets. Thinning was done seven (7) days after sowing, to have two (2) plants per pocket.

2.4 Data collection and statistical analysis

Five (5) plants were randomly selected in each plot at the beginning of flowering stage to assess nodulation, plant height and biomass. The plants height were measured with a tape measure. To assess nodulation, plants were carefully uprooted by digging 20 cm around the plant. Roots were washed delicately with clean water to remove all attached soils from root and nodules. Nodules were carefully detached from root, counted and oven-dried at 60° C for 72 h.

Plants wet biomass were measured and oven-dried at 70 °C for 72 h to their dry weight recorded.

At maturity, the average number of pods per plant was counted from five randomly selected plants.

Seed yield of soybean (kg ha^{-1}) was measured from each plot and converted in hectare base.

Statistical analysis were conducted using standard statistical procedures. Data relating to soybean nodulation, growth and yield parameters were collected. These data were firstly checked for meeting all analysis of variance (ANOVA) assumptions for normality and of distribution and homogeneity of variance. They were secondly subjected to ANOVA using STATISTICA 7.1 version when ANOVA assumptions are verified. Fisher's Least Significant Difference test (LSD) was used to separate means at a significance level of 5 %.

3. RESULTS AND DISCUSSION

3.1 Results

3.1.1 Effects of local rhizobia strains on soybean nodulation

The number of nodules varied significantly ($P = 0.02$) between treatments (Figure 1). Observation of the root system showed that the non-inoculated controls (fertilized and non-fertilized) had no nodules on their roots. On the other hand, all the bacteria used as inoculum induced nodule formation on the root system of this cultivar. The number of nodules per plant varied from 23 to 34. The highest number of nodules (34) was observed on the root systems of plants inoculated with the local bacteria strains RSC324 and RSC502. These bacteria had similar effects on nodule formation with the exotic strain IRAT FA3. The latter induced the same effects as local rhizobia strains RSC119 and RSC508 with 32, 30 and 31 nodules per plant respectively. Local nitrogen fixing bacterium strain RSC309 was the least efficient.

In the same study, nodules dry weight varied according to the bacterium (Figure 1). The bacterial strain RSC324 induced the highest nodule weight at 111 mg compared to all the bacteria tested. Also, the reference strain IRAT FA3 statistically induced the same nodule weight as the other isolates tested, with the exception of bacterium strain RSC115. Nodules from plants inoculated with the latter strain had the lowest dry nodule weight (44 mg).

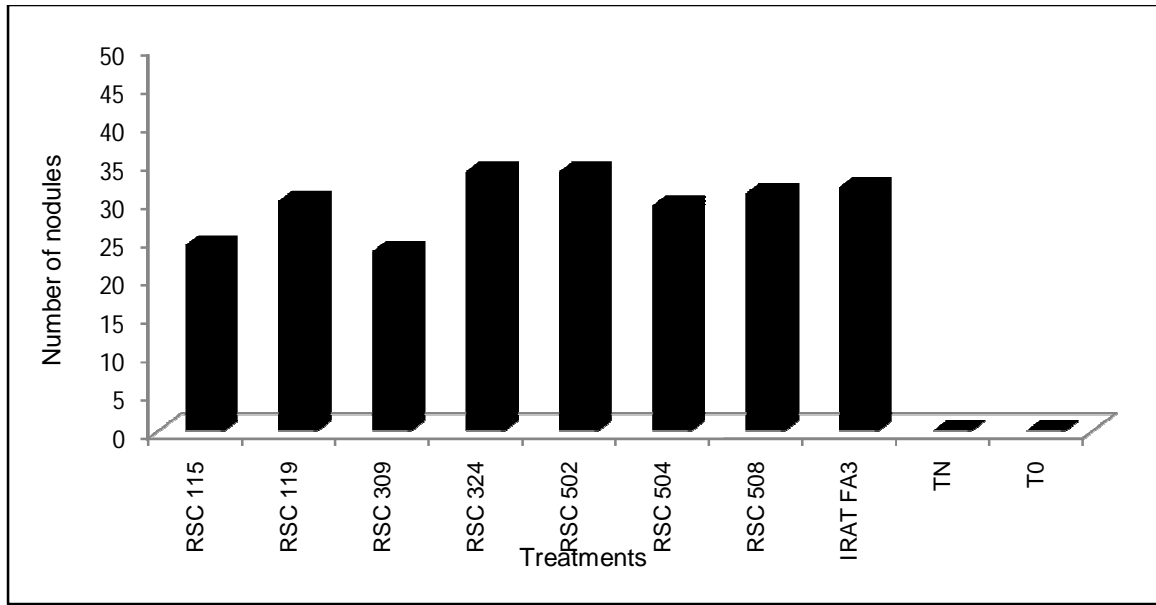


Figure 1 : Effect of inoculation on the number of nodules formed on soybean cultivar Canarana

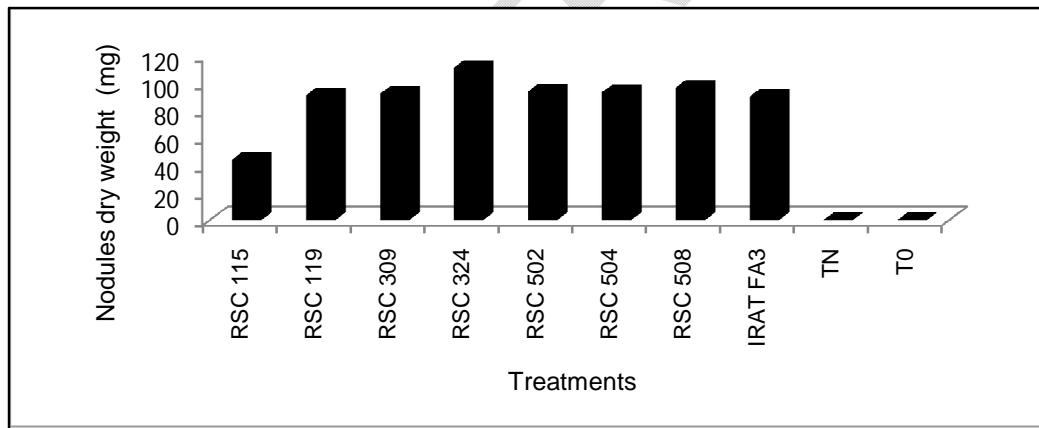


Figure 2 : Effect of inoculation on dry weight of nodules formed on soybean cultivar Canarana

3.1.2 Effects of local rhizobia strains on soybean height and biomass production

All tested bacteria significantly ($P = 0.02$) increased the height of the soybean plants compared with the uninoculated and unfertilized control (T0). Plants inoculated with the local bacterium strain RSC502 showed the greatest height (38.40 cm). In addition to this strain, the other local bacterial strains RSC324, RSC504, RSC508 and RSC309 induced soybean height higher than the introduced strain IRAT FA3 (37.60 cm). However, none of the treatments increased plant height more than the fertilized control (41.80 cm).

The tested bacteria also had a significant effect ($P = 0.02$) on soybean fresh biomass production at the beginning of flowering. All bacteria increased the fresh biomass of plants of the Canarana cultivar compared with the absolute control (Table 2). The highest fresh biomass was obtained with fertilized plants (16.5 g). The effects of this synthetic fertilizer on fresh biomass production were statistically

similar to those of the local rhizobia strains RSC309 (15.3 g), RSC324 (15.8 g), RSC502 (15.5 g), RSC504 (15.1 g) and the introduced strain IRAT FA3 (15.1 g).

All the tested bacteria had a significant influence ($P = 0.005$) on soybean dry biomass. The dry biomass induced with the local bacteria was found to be better than that of untreated plants. The local strains RSC309 (3.4 g), RSC324 (3.3 g), RSC502 (3.3 g) and RSC508 (3.3 g) increased dry biomass compared to the uninoculated and unfertilized control T0 (2.8 g). However, plant dry weights induced by local bacteria were lower than those induced by synthetic fertilizer (TN) with 3.9 g (Table II).

Table II : Effect of local rhizobia, introduced strain and NPK 12-22-22 fertilizer on height and biomass of soybean cultivar Canarana

Treatments	Plant height (cm)	Fresh biomass (g)	Dry biomass (g)
RSC115	33±1d	12.5±1.2c	3±0.6bc
RSC119	35.80±0.8c	13±0.6c	3±0.4bc
RSC309	36.80±1.3bc	15.3±1.5ab	3.4±0.21b
RSC324	38.20±1.5b	15.8±1.4ab	3.3±0.3b
RSC502	38.40±1.1b	15.5±0.7ab	3.3±0.3b
RSC504	37.60±1.5b	15.1±0.8ab	3.1±0.3bc
RSC508	37.40±1.3bc	14.9±1.3b	3.3±0.3b
IRAT FA3	37.60±1.1b	15.1±1.5ab	3.2±0.4bc
TN	41.80±1.3a	16.5±0.6a	3.9±0.3a
T0	32±1.6d	12.29±1.1c	2.8±0.3c
Mean	36.9±2.9	14.6±1.7	3.2±0.4
CV (%)	7.9	11.9	12.7
LSD (5 %)	1.8	1.5	0.5

In each column, the means followed by the same letter are not significantly different at the 5 % level according to the Fisher LSD test. T0: Untreated control; TN: NPK 12-22-22 synthetic fertilizer.

3.1.3 Effects of local rhizobia strains on soybean yield and yield components

All local rhizobia inoculated to soybean had a significant influence ($P = 0.04$) on the number and weight of pods (Table III). Rhizobia strains RSC324 and RSC502 induced more pods than the other bacteria. These rhizobia induced 144 and 133 pods per plant respectively. In addition to these strains, strain RSC508 induced more pods per plant than the introduced strain IRAT FA3 and the synthetic fertilizer 12-22-22 formulation. Pods weight was also increased with all the tested rhizobia. Bacteria strains RSC324 and RSC508 induced the highest pods weight (55.7 and 52.2 g) respectively. The pods weight obtained with these local bacteria were higher than those recorded with the IRAT FA3 strain (42.9 g) and the chemical fertilizer (51.3 g).

One hundred (100)-seed weight and seed yield per hectare were also significantly influenced by inoculation ($P = 0.03$). The weight of 100 seeds varied from 10.1 g to 15.5 g. The highest 100-seed weight were obtained with bacteria strains RSC309 and RSC119. These bacteria induced 15.2 g and 15.5 g respectively. They significantly increased this parameter compared with the introduced bacterial strain IRAT FA3 (13.7 g) and the chemical fertilizer formulation 12-22-22 (14.3 g). Seed yield was also improved by the rhizobia tested compared with the unfertilized control. This yield varied from 0.5 t.ha⁻¹ to 2.5 t.ha⁻¹. The yield recorded on the control plot was 0.5 t.ha⁻¹. The yield was improved more with rhizobia strains RSC324 and RSC502 with 2.5 t.ha⁻¹. The yields induced by these bacteria were higher than those obtained with synthetic fertilizer (2.4 t.ha⁻¹) and the introduced strain IRAT FA3 (2.3 t.ha⁻¹). Furthermore, the yield obtained with the application of chemical fertilizer was statistically similar to those obtained with local nitrogen fixing bacteria RSC504 and RSC508.

Table III : Effect of local rhizobia, introduced strain IRAT FA3 and chemical fertilizer NPK 12-22-22 on yield parameters of soybean cultivar Canarana

Treatments	Pods number.plant ⁻¹	Pods weight.plant ⁻¹ (g)	100-seeds weight (g)	Seeds yield (t.ha ⁻¹)
RSC115	91±5g	35±2.1d	13.6±0.2h	1.9e
RSC119	108±10ef	44.8±5.3c	15.2±0.3b	2.2d
RSC309	112±5def	50.3±2.2b	15.5±0.1a	2.3c
RSC324	144 ±17a	55.7±3.4a	13.8±0.1fg	2.5a
RSC502	133±9ab	49.6±2.8b	14±0.2de	2.5a
RSC504	118±6cde	50.7±2.2b	14±0.1ef	2.4b
RSC508	129±8bc	52.2±2.2ab	14.2±0.1cd	2.4b
IRAT FA3	102±8fg	42.9±2.4c	13.7±0.1gh	2.3c
TN	120±8cd	51.3±3.5b	14.3±0.1c	2.4b
T0	51 ±3h	24.5±2.3e	13.7±0.0gh	0.5±0.1f
Mean	111±26	45.7±9.5	14.2±0.6	2.1
CV (%)	23.5	20.8	4.6	24.7
LSD (5 %)	11	4.4	0.2	0.1

In each column, the means followed by the same letter are not significantly different at the 5 % level according to the Fisher LSD test. T0: Untreated control; TN: NPK 12-22-22 synthetic fertilizer.

3.2 Discussion

Soybean is a recently introduced crop in Divo area. It therefore needs competitive rhizobia inoculum to boost its productivity. The choice of such bacteria must take into account environmental conditions to enable them to express their potential. Thus, seven (07) local rhizobia (RSC115, RSC119, RSC309, RSC324, RSC502, RSC504 and RSC508) were tested on farm conditions in Divo to assess their ability to adapt to this zone. The results showed that local rhizobia tested were capable of significantly improving the various parameters studied.

All local bacteria induced the formation of nodules on the soybean root system. Among the tested bacteria, local rhizobial strains RSC324 and RSC502 induced the higher nodules number with the corresponding dry weight. These bacterial strains are thought to be more competitive than endogenous bacterial strains and better adapted to local environmental conditions than other bacteria [3]. However, for some authors, the competitiveness of local bacterial strains depends on their compatibility with the soybean genotype grown. This is the case of Habibi *et al.* [11], who showed that indigenous strains of *Ensifer* (GS4 and GE6W) and *Bradyrhizobium* (GE3) induced the formation of more nodules on the Stime3300 and Enrei soybean varieties compared with the USDA110 reference strain in Kabul province in Afghanistan. Also, variation on nodules formation between local rhizobia strains tested could be explained by a difference in the ability of these bacterial strains used as inoculum to form functional nodules on soybean roots [12]. In the current study, no nodules were observed on the roots of uninoculated plants (plants fertilized with NPK and untreated plants). Results suggest that no existing effectiveness native rhizobia in experimental site soil or their population is not adequate to induced symbiosis [13].

In terms of soybean growth, parameters such as height, fresh and dry biomass were improved by the bacteria tested compared to the unfertilized control. Plant height and biomass improvement observed in this work is thought to be due to the nitrogen supplied during symbiotic fixation of the atmospheric nitrogen. Alam *et al.*[14]reached the same conclusion in Bangladesh. These authors showed that height and dry biomass of soybean genotypes Shohag, BARI Soybean6, MTD10 and BGM02026 were improved by the local *Rhizobium sp.*strain BARI Gm901 compared to the uninoculated control. Also, the tested bacteria generally induced the same effects as the synthetic fertilizer 12-22-22 formulation on height and biomass production of soybean. Ntambo *et al.* [15]attributed soybean growth improvement to fertilizer use.

Soybean inoculated with the different bacteria showed an increase in the number and weight of pods compared to the controls. Among the bacteria tested, RSC324 and RSC502 produced more pods than the synthetic fertilizer NPK 12-22-22. The highest number of pods (144) was observed on the plots inoculated with the local nitrogen fixing bacterium strain RSC324. In addition to these strains, other local strains such as RSC504 and RSC508 induced similar effects on pod production as the synthetic fertilizer. According to Abdel-Fattah *et al.*[16], the inability of synthetic fertilizer to increase the number and weight of pods compared with inoculation is due to its low soil application. Other researchers suggested that soybean plants inoculated with rhizobial strains may produce statistically similar numbers of pods to those fertilized with synthetic fertilizers [17].

100-seeds weight and seeds yield per hectare were also improved by inoculation compared with the untreated control. Higher 100-seeds weights were obtained with the local bacteria RSC309 and RSC119. Also, all bacteria increased soybean seeds yield per hectare compared to the control fertilized with chemical fertilizer 12-22-22 formulation. The highest seeds yield ($2.5 \text{ t} \cdot \text{ha}^{-1}$) was induced by the local strains RSC534 and RSC502. Patra *et al.* [18] achieved similar results with soybean cultivar PK-416 in India. According to Solomon *et al.*[19], the higher seed yield may be attributed to the most pods number induced by local bacteria strains. On the other hand, other works attributed the performance of rhizobia on soybean yield to the relationship between nodules formation and root weight[21 ; 22]. However, Shahid *et al.*[20]concluded that the variation in soybean yield is largely due to the combined effect of bacterial strains on vegetative and production parameters, which in turn are influenced by environmental conditions. For other authors, soybean seed yield increasing with rhizobial strains is linked to the low nitrogen content of tropical soils. This low nitrogen content in the soil is at the origin of the initiation of symbiosis between the soybean and its symbiont [23 ; 24].

4. CONCLUSION

Local rhizobia strains RSC324 and RSC502 induced the most nodules on the roots of soybean cultivar Canarana at Divo. These same bacteria induced more pods per plant than the introduced bacterial strain IRAT FA3 and synthetic fertilizer (12-22-22 formulation). The highest seeds yield was obtained with local bacterial strains RSC324 and RSC502. In addition to these bacteria, the local **strain** RSC508 increased soybean pods yield compared with synthetic fertilizer. As a result of this study, the local rhizobia RSC324 and RSC502 proved to be the most effective and competitive for soybean cultivar Canarana in Divo. These bacteria are therefore highly recommended for the sustainable management of small farms in Divo area.

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Option 1:

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc.) and text-to-image generators have been used during the writing or editing of this manuscript.

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