

Full Length Research Paper

Effect of inoculating seeds with *Bradyrhizobium japonicum* on the agronomic performance of five varieties of soybean (*Glycine max*) in Côte d'Ivoire

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Recent studies in the Nawa region of Côte d'Ivoire have indicated an acute malnutrition rate of 11.3% among cocoa producers. One of recommended actions from the studies was to diversify agriculture with nutrients rich crops. Introduction of soybean (*Glycine max*) cropping system could go a long way to ensure food and nutritional security in the region. The current study was conducted in two sites (Logboayo and Soubré) in the south-west of Côte d'Ivoire, to evaluate the effect of IRAT-FA3 *Bradyrhizobium japonicum* strain inoculum on the agronomic performance of five varieties of soybean named Doko, Canarana, V3_2013, V6_2013 and IT_235. The experimental design was randomized complete block with a split plot with inoculation as the main factor and variety of soybean as subplot treatment replicated three times. Data were collected on some yield parameters and the grain yield. Results of yield showed a highly significant effect ($P < 0.0001$) of the site and a significant effect ($P = 0.0316$) of the variety x treatment interaction. Highest yield was recorded at Logboayo with 1838 kg ha⁻¹ compared to 1220 kg ha⁻¹ for Soubré. The variety V6_2013 with a yield of 1931 kg ha⁻¹ and good vegetative development could be recommended as elite variety for the farmers in the Nawa region.

Key words: Soybean, Côte d'Ivoire, agronomic performance, yield, variety, inoculation.

INTRODUCTION

Soybean (*Glycine max* L. Merrill) is an annual herbaceous plant of the family of Fabaceae and native to eastern Asia. It is a legume cultivated for its seeds which are highly rich in protein and oil (FAO, 1995; Nyabyenda, 2005). Its seeds contain the highest protein content of all food crops. Soybean comes in second position for the amount of oil after peanut for legumes (Gurmu et al., 2009). This richness in fat and vegetable protein allow

soybean to be a popular foodstuff in food and feed (FAO, 1995; Pirot, 1998). This crop can use atmospheric nitrogen through biological fixation by establishing a symbiotic relationship with Rhizobium bacteria (Vossen, 2007). Indeed, *Bradyrhizobium japonicum* is a nitrogen-fixing soil bacterium that has a symbiotic relationship with the host legume soybean *Glycine max* (Lee et al., 2012). Such symbiosis makes it less dependent on soil nitrogen

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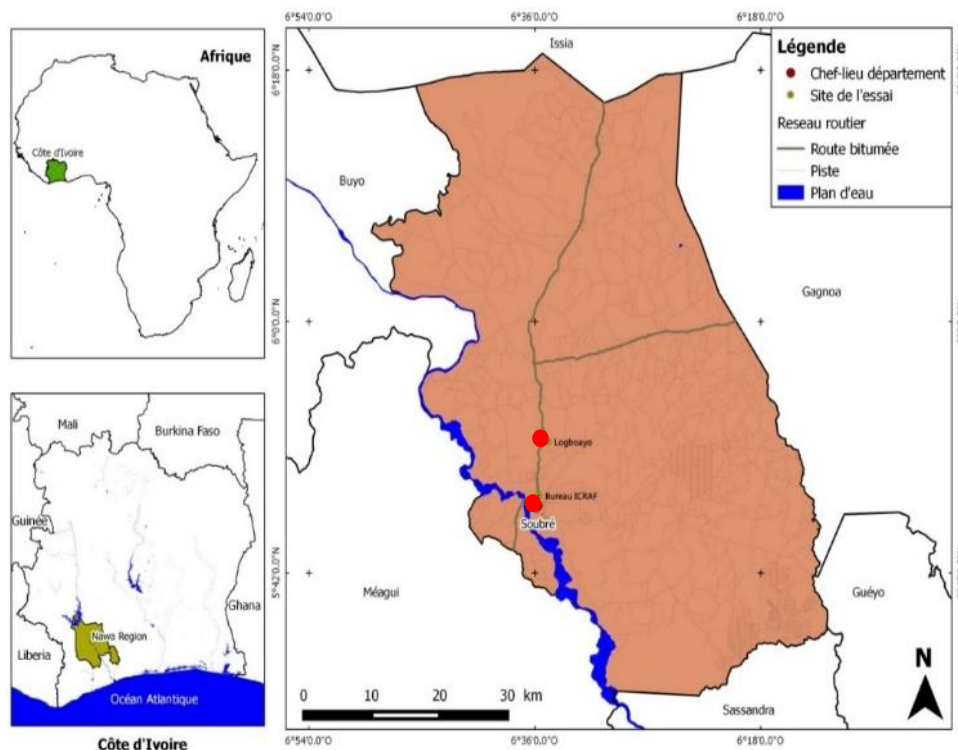


Figure 1. Location of the study area.

particularly in tropical regions of Africa where soils are deficient in nitrogen (FAO, 1995). Given this potential, soybean is considered as a strategic plant for producing countries according CIRAD (2002).

In Côte d'Ivoire, soybean farming was developed in the 1980s by the policy of diversification of food crops in the country (Chaléard, 1996; Beugré et al., 2013). The first results of experiments in the soybean project in the Northwest showed significant potential of soybean (N'gbesso et al., 2013). The national production of this crop is less than 6000 tons, and this quantity is insufficient to satisfy the needs of the Ivorian population estimated at 20 000 tons (CNRA, 2002; N'zoué et al., 2003). In addition, the adoption of the soybean crop is hindered by the lack of suitable variety really adapted to the environmental conditions in the respective areas (N'gbesso et al., 2009; Ama-Abina et al., 2012; Tukamuhabwa et al., 2012). Therefore, the search for varieties adapted to each agro-ecological zone is a precondition for increasing the yield of production and ensure food and nutrition security (Nieuwenhuis and Nieuwelink, 2005).

The region of NAWA contributes to 20% of cocoa production in Côte d'Ivoire (MINAGRI, 2009). The development of cocoa-farming in this region has been at the detriment of food crops which has consequently caused a food crisis in the region, resulting in the shortage of major commodities in the local market. Most

households are indeed in a situation of food insecurity and vulnerability with a prevalence of 21.5% (Coulibaly, 2013). In addition, there is limited information available on soybean in this region; despite the opportunities that crop could offer to the people of the Nawa region.

To ensure food and nutrition security in this region, initiatives are being undertaken to diversify crops with the introduction of soybean in the area of the Nawa and, to facilitate its adoption. This work is part of a wider program. It is in this context that this study was conducted to investigate the agronomic performance of five soybean varieties in order to select the most suitable. The objective of this study was to compare the agronomic performance of different varieties of soybean in Soubré and Logboayo in the Nawa region of Côte d'Ivoire.

MATERIALS AND METHODS

Study site

Experiments were performed from September 2014 to February 2015 at the World Agroforestry Centre (ICRAF) Stations in Logboayo and Soubré, located in the Nawa Region; South-West of Côte d'Ivoire, 5°47'08"N, 6° 36'30"W, 276 m a.s.l. (Figure 1).

The vegetation is evergreen with a fraction of dense rainforest semi-deciduous or mesophilic (Kouamé and Zorobi, 2010). The type of sub-equatorial climate is characterized by the existence of two rainy seasons and two dry seasons. The heaviest rains are experienced in June. The mean annual rainfall is 1360 mm and

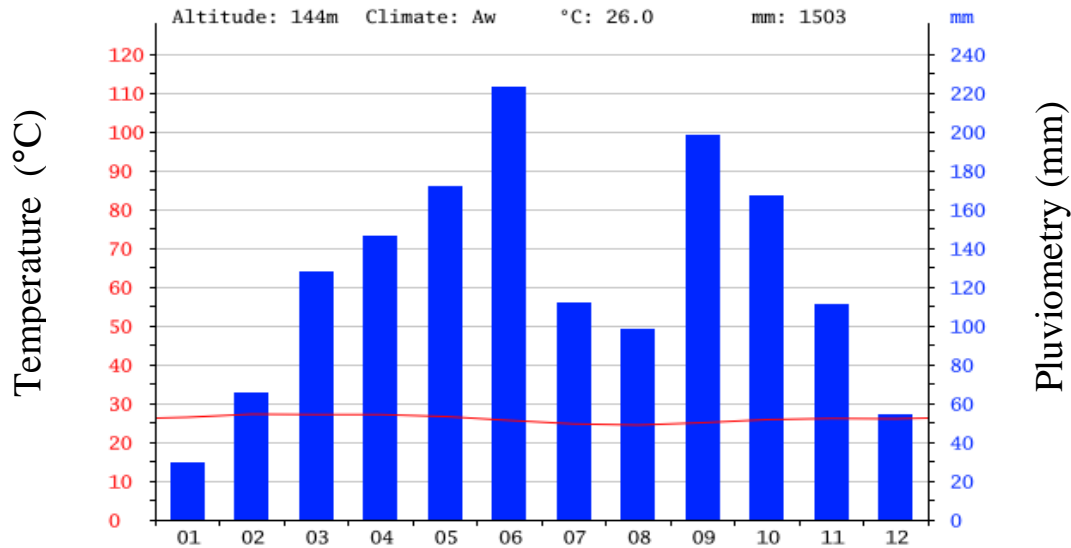


Figure 2. Ombrothermic diagram of the city of Soubré in 2013. Source : <http://fr.climate-data.org/location/58795/>.

varies from 968 to 1767 mm. The soils are classified as Ferralsols and Gleysols, generally acid, subject to leaching and chemically poor (De Rouw, 1994). The minimum and maximum temperature varies from 23 to 36°C (Figure 2).

The trials were conducted in two sites, one in Soubré and the other at Logboayo Station located 9 km from Soubré. The land in the two sites was previously planted with yams. The experimental plots were located on the top of a slope gradient of 9 and 7%, respectively, for Soubré and Logboayo. The pH of soil at Logboayo was 5.25 and 5.5 for Soubré.

Plant material

Five soybean varieties named Canarana, Doko, V3_2013, V6_2013 and IT_235 were used for the study. They were popularized in Côte d'Ivoire by the Soybean project for over 20 years (N'gbesso et al., 2010). Seeds used in this study were provided by the Research Station on Food Crops of the National Agricultural Research Centre (CNRA) in Bouaké.

Strain of bacterium

The IRAT-FA3 *Bradyrhizobium japonicum* strain was used for inoculum production. The strains of this species, known for a perfect symbiosis with soybean, are insignificant or absent in tropical soils (Cattelan and Hungria, 1995). Peat was used as inoculum substrate. It is described as being very competitive in acid soil and has already been used in Rwanda, Burundi, Madagascar, Cameroon and Côte d'Ivoire (Beugré et al., 2013). The inoculum was produced and provided by the Inoculum Production Unit for Legumes (UPIL) of the CNRA in Bouaké, Côte d'Ivoire.

Experimental design

All experiments were laid out in a randomized complete block design with split plots arrangement replicated three times with two factors in each site. The main factor was the inoculation with two levels: Inoculated treatment and uninoculated treatment or control.

The second factor was the soybean variety, with five levels (Canarana, Doko, V3_2013, V6_2013 and IT_235). A block or replicate was divided into two sub-blocks or large plot. Each large plot was assigned with a treatment (Inoculated or uninoculated). Each large plot was divided into as many elementary plots as soybean varieties.

The size of the sub plots was 4 × 3 m giving a total area of 12 m². Two consecutive blocks were separated by a space of 2 m wide. Distance between 2 elementary plots of the same block was 1 m. A space of 2.5 m was left between the borders of the field and the blocks. The elementary plot of 12 m² was represented by 7 lines of 4 m seedlings, each. Data was collected from 5 lines on the inside of the basic plot 0.5 m from each side, therefore on an area of 6 m². There seeds per hole were sowed at 50 cm spacing between the rows and 20 cm between the holes of the lines seed. The experimental plot had a total of 12600 plants.

The site was cleared and this was followed by a manual deep plowing of 30 cm and making ridges. A fertilizer NPK12-24-18 was applied at the rate of 100 kg ha⁻¹. No pesticide treatment was applied throughout the crop cycle. In addition, three manual weeding and tilling were made on the 15th, 30th and 55th days after sowing (DAS) to aerate the soil and reduce competition with weeds. The harvest was done gradually following the period of maturity of the different varieties.

Inoculation of seeds

Seeds were inoculated with the *Bradyrhizobium japonicum* bacterium before being sowed at a depth of 3 cm (Kouamé et al., 2007). The broth method or inoculation by seed coating method was used (Vitosh, 1997). The proportion was 100 g of inoculum for 15 kg of seed. The bacteria density was 10⁹ bacteria/g of inoculum (N'gbesso et al., 2010). The control plots were sowed first in order to avoid contamination by bacteria of the inoculum.

Data collection

Data were collected on pod maturation period, plant density at harvest, dehiscence rate at harvest, height of the first pod insertion,

Table 1. Effect of inoculation on pod maturity period, plant density at harvest, number of fruiting nodes, insertion height of first pod and height at harvest of the soybean varieties

Variety	Pod maturity period (DAS)	Plant density at harvest (%)	Number of fruiting nodes	Insertion height of first pod (cm)	Height at harvest (cm)
Canarana	100.30 ^{d*}	55.81 ^{bc}	10.42 ^b	5.77 ^a	29.17 ^c
Doko	97.40 ^c	53.78 ^{bc}	8.25 ^a	9.58 ^c	37.17 ^a
IT_235	95 ^b	62.85 ^a	11.10 ^b	7.83 ^b	35.56 ^a
V3_2013	88.72 ^a	52.68 ^b	11.02 ^b	6.40 ^a	31.42 ^b
V6_2013	103.53 ^e	23.95 ^c	10.56 ^b	5.67 ^a	31.11 ^b
Means	96.94	50.09	10.26	7.19	32.94
CV (%)	5.34	44.16	19.77	44.93	20.16

*Means followed by the same letter are not significantly different at $p > 0.05$.

plant height at harvest, number of fruiting nodes, pods per plant, seed per plant, and seed per pod, pod filling rate, weight of 100 seeds and grain yield of soybean varieties.

Data analysis

All data were subjected to two way analysis of variance (ANOVA), using the general linear model of Statistical Analysis System (SAS) software (SAS, 2003), and the significance difference between the means were assessed with Duncan method at 5%.

RESULTS AND DISCUSSION

Table 1 shows the results of the most important agronomic characteristics of soybeans. The pod maturation process began about 77 days after sowing (DAS) with V3_2013 variety. A highly significant difference was observed among the soybean varieties ($P < 0.0001$). The pod maturity period for this variety was reached about 88 DAS. V3_2013 was followed by IT_235 and Doko varieties with the pod maturity periods of 95 and 97 DAS, respectively. Varieties Canarana and V6_2013 expressed the longest pod maturity period with 100 and 103 DAS respectively (Table 1).

The varieties being evaluated registered good plant density at the harvest except V6_2013. The highest plant density was shown by the variety IT_235 with about 63 plants. Then, Canarana, Doko and V3_2013 had between 52 and 56 plants. The lowest density was observed with the variety V6_2013 which had 23 plants (Table 1). Analysis of variance showed a highly significant difference among the soybean varieties and the treatment ($P < 0.0001$). The interaction of Treatment x variety was significant ($P = 0.0132$).

The average number of fruiting nodes on the main stem ranged from 8.25 to 11.02. Analysis of variance showed a significant difference among the soybean varieties. Varieties were classified into two groups according to the number of nodes. The first group consists of varieties Canarana, IT_235, V3_2013 and V6_2013. These varieties with 11 nodes had the largest numbers of nodes compared to the variety Doko, of the second group with

8.25 nodes (Table 1).

Concerning the height of insertion of the first pods, 3 groups were distinguished. The highest height was observed in the Doko variety with 9.58 cm. It was followed by the IT_235 variety with 7.53 cm. Canarana, V3_2013 and V6_2013 varieties, the 3rd group, expressed the lowest heights with 5.77, 6.4 and 5.67 cm, respectively (Table 1).

Variance analysis identified a highly significant effect of treatment on dehiscence rate of the seeds. Indeed, the inoculated plants with dehiscent seeds were low compared to non-inoculated plants with averages of 2.37 and 4.99, respectively. In addition, a variety effect was observed in both inoculated plants and the control. Varieties Canarana and V6_2013 had high dehiscence rate for both treatments. The varieties Doko and IT_235 had a very small number of plants with dehiscent pods. No dehiscent pod was observed in the V3_2013 variety (Table 2).

The analysis of variance on the pod filling rate showed no significant effect of the two factors and their interaction and among the soybean varieties. However, the inoculated plants had significantly slightly higher fill rate than non-inoculated plants. These rates ranged from 77.40 to 90.94% for inoculated plants and 52.69 to 88.38% for controls (Table 2).

The results showed a highly significant effect of inoculation, variety and the treatment x variety interaction on the number of pods per plant ($P = 0.0016$ and $P < 0.0001$). Inoculation contributed to a significant increase in the number of pods per plant. The number of pods of Canarana variety increased from 47.85 in the control to 74.74 in the inoculated. For the variety Doko, the number of pods per plant increased from 37.27 to 41.12. In IT_235; this variable ranged from 43.40 to 65.56 and for V6_2013 from 59.48 to 67.79. However, the variety V3_2013 was observed to have a high number of pods per plant in non-inoculated plants than in plants inoculated with 69.10 and 52.80 (Table 2).

The number of seeds per plant of the soybean varieties was improved by the inoculation except for V3_2013.

Table 2. Effect of inoculation on dehiscence rate, pod filling rate and number of pods per plant of 5 soybean varieties.

Variety	Dehiscence rate (%)		Pod filling rate (%)		Number of pod per plant	
	I1	I0	I1	I0	I1	I0
Canarana	9.39 ^{a*}	13.72 ^a	77.40 ^a	52.69 ^a	144.64 ^a	92.46 ^b
Doko	0 ^b	1.99 ^c	76.29 ^a	67.24 ^a	93.66 ^b	84.00 ^b
IT_235	0.55 ^b	0.47 ^c	85.44 ^a	86.27 ^a	137.30 ^a	89.36 ^b
V3_2013	0 ^b	0 ^c	90.94 ^a	88.38 ^a	106.88 ^b	136.14 ^a
V6_2013	1.64 ^b	8.55 ^b	77.35 ^a	74.57 ^a	148.79 ^a	128.91 ^a
Means	2.37	4.99	79.97	69.58	125.70	106.02
CV (%)	275.16	208.28	14.01	86.17	46.19	53.59

*Means followed by the same letter are not significantly different at $p > 0.05$.

Table 3. Effect of inoculation on the number of seeds and the weight of 100 seeds of the soybean varieties.

Variety	Number of seeds		Weight of 100 seeds (g)	
	I1	I0	I1	I0
Canarana	1.97 ^{a*}	1.91 ^a	11.08 ^c	10.06 ^c
Doko	2.25 ^a	2.26 ^a	14.26 ^a	13.40 ^a
IT_235	2.10 ^a	2.05 ^a	12.16 ^{bc}	10.80 ^b
V3_2013	2.03 ^a	1.96 ^a	11.74 ^{bc}	11.98 ^{ab}
V6_2013	2.20 ^a	2.16 ^a	13.68 ^{ab}	12.28 ^{ab}
Means	2.11	2.06	12.58	11.70
CV (%)	10.31	11.42	13.93	14.53

*Means followed by the same letter are not significantly different at $p > 0.05$.

Canarana, Doko, IT_235 and V6_2013 recorded a respective increase in the number of seed of 52.18, 9.66, 47.91 and 19.88. The control V3_2013 had 136.14 seeds compared to 106.66 for treatment with inoculation. Regarding the number of seeds per pod, inoculated and non-inoculated plants had an average of 2.11 and 2.06 seeds respectively. There was no effect of the treatment and the variety (Table 3).

According to the weight of 100 seeds, it was noted a net improvement by the inoculation with an average of 12.58 g as opposed to 11.70 g for the control. Furthermore, in the same treatment, a highly significant effect of the variety was observed. Doko variety had the highest weight of 100 seeds in both treatments with 14.26 g for inoculated plants and 13.40 for the control. It is followed by V6_2013, IT_235 and V3_2013. Lowest weight was obtained by Canarana variety in each treatment with 11.08 and 10.06 g, respectively in the inoculated and in the control (Table 3).

The pod maturation period revealed that varieties having obtained a good nodulation were early with homogeneity in the maturation of pods. This could mean that the ability to nodulation appearance of varieties did intervene in the maturation process. Indeed, for the varieties IT_235 and V3_2013, harmonious browning pods (Fehr and Caviness, 1977) allowed obtaining a low

rate of dehiscence. In contrast, late varieties Canarana and V6_2013 had a heterogeneous maturation manifested by the presence on the same plant of ripe and not ripe pods. This has resulted in a high loss by seeds dropping on feet of these two varieties with losses per basic plot about 69.1 and 26.37%, respectively.

For the number of pods per plant, the pods filling rate, the number of seeds per pod and the weight of 100 seeds, inoculation led generally to the improvement of these characters in the inoculated plots. Indeed, in these plots, plants have the atmospheric nitrogen fixed by symbiotic bacteria in addition to the nitrogen in the soil. These studies concur with those of Ama-Abina et al. (2012) and Ngbesso et al. (2013). According to Gazzoni (1995), atmospheric nitrogen is easily usable by soybean and goes directly into the formation of fruiting bodies, such as pods and seeds in relation to the nitrogen from the soil (Cattelan and Hungria, 1995). These results will allow selecting the best varieties for the locality.

Grain yield

Inoculation improved grain yield of soybean varieties. Highly significant effects of the site and variety x treatment interaction were observed on this parameter

Table 4. Effect of inoculation on grain yield of the soybean varieties

Variety	Logboayo (kg ha ⁻¹)		Soubré (kg ha ⁻¹)		Whole study (kg ha ⁻¹)	
	I0	I1	I0	I1	I0	I1
Canarana	1864.0 ^{a*}	2250.5 ^a	929.0 ^b	1450.7 ^a	1303.0 ^a	1770.6 ^a
Doko	1912.0 ^a	2231.0 ^a	1023.7 ^b	1334.3 ^{ab}	1379.0 ^a	1699.0 ^a
IT_235	1626.0 ^a	1821.0 ^a	1448.3 ^a	1196.7 ^{ab}	1519.4 ^a	1446.4 ^{ab}
V3_2013	1662.0 ^a	1337.0 ^a	1102.7 ^{ab}	755.7 ^b	1326.4 ^a	988.2 ^b
V6_2013	1601.5 ^a	2079.0 ^a	1114.7 ^{ab}	1833.3 ^a	1309.4 ^a	1931.6 ^a
Means	1733.1	1943.7	1124.7	1316.1	1367.4	1567.2
CV (%)	24.30	27.61	18.38	25.20	21.64	26.25

*Means followed by the same letter are not significantly different at $p > 0.05$.

with $P < 0.0001$ and $P = 0.0316$, respectively. Indeed, the grain yield obtained was higher at Logboayo (1838 kg ha⁻¹) compared to Soubré (1220 kg ha⁻¹) for all the soybean varieties and the treatment (Table 4).

At Logboayo, no significant difference was observed among varieties of inoculated and the control. Grain yield of Canarana ranged from 1864 to 2251 kg ha⁻¹ with an increase of 387 kg ha⁻¹ due to the inoculation. For Doko, the grain yield oscillated in 1912 to 2231 kg ha⁻¹ with an increase of 319 kg ha⁻¹. Variety IT_235 registered 1626 to 1821 kg ha⁻¹, 195 kg ha⁻¹ of increase. For V3_2013 the control gave a grain yield of 1662 kg ha⁻¹ compared to 1337 kg ha⁻¹ for the treatment.

At Soubré, significant effects of the variety and the variety x treatment interaction were observed on the yield with $P = 0.0356$ and $P = 0.0106$, respectively. An improved performance by inoculation was also observed. The three varieties V6_2013, Canarana and Doko recorded a yield increase of 718, 522 and 310 kg ha⁻¹, respectively. However, for varieties IT_235 and V3_2013, the grain yield of the control was higher than that of the inoculated treatment. This could be probably because these varieties did not nodulate with the strain *Bradyrhizobium japonicum*.

Regarding the grain yield per hectare, inoculation improved this parameter for the inoculated plants. The best yields were obtained at Logboayo with an estimate of 2251, 2231 and 2079 kg ha⁻¹ for varieties Canarana, Doko and V6_2013, respectively. At Soubré, the best variety was V6_2013 with 1833 kg ha⁻¹. It is followed by Canarana and Doko with 1451 and 1344 kg ha⁻¹, respectively. Variety V3_2013 exhibited a decrease of its yield regarding inoculated compared to uninoculated controls in Soubré and Logboayo. This is a good justification of nodulation ability of this variety with indigenous rhizobia. This work confirmed a spontaneous nodulation induced by indigenous strains of bacteria.

During the current study, V6_2013 with a yield of 1932 kg ha⁻¹ and an increase of 622 kg ha⁻¹ compared to the control, followed by Canarana with a yield of 1770.6 kg ha⁻¹ and an increase of 467 kg ha⁻¹ and Doko (1699 kg ha⁻¹) with an increase of 320 kg ha⁻¹ were the best

varieties. The variety V6_2013 got a low grain yield, also in the inoculated treatment than the control. This was certainly due to its low plant density observed in the two tests (N'gbesso et al., 2013). In addition, it was noted an early drying of plant in the maturation phase. This has therefore intensified the dehiscence rate and the loss by dropping of seeds. The best performance obtained in Logboayo could be explained by two successive rains occurred in Logboayo during pod filling when plants in Soubré were manually irrigated during this critical time when they needed water. This irregularity of rainfall was mentioned by Ama-Abina et al. (2012) to justify the low yields obtained in some trials of soybean cultivation in Gagnoa, Côte d'Ivoire. In fact, soybean has two critical periods of water requirement: to plant emergence and timing of pod filling (FAO, 1995).

According to Anthony (2005), a typical inoculation of soybean seeds induces an increase of bacteria in the soil by 256.10⁹ bacteria per plant are in the soil. The average yields of 1838 kg ha⁻¹ in Logboayo is lower than those obtained by N'gbesso et al. (2010) to 3260.68 kg ha⁻¹ in Bouaké and 3560.75 kg ha⁻¹ in Touba, but higher than those obtained by Nzabi et al. (2000) with two varieties; Congo Belgium (1720 kg ha⁻¹) and Hill (1430 kg ha⁻¹) in the South-west of Kenya after inoculation. Result of 1220 kg ha⁻¹ in Soubré is lower than what these authors obtained. Moreover, the results of this study also showed that the improved performance and other parameters studied after inoculation varied according to the locality and the varieties tested. Indeed, differences between yields of different localities, induced by inoculation were also reported by Yen (2004) in Vietnam. These different behaviors are linked to the ability of each variety to fix nitrogen with the strains of bacteria in presence (Gwata et al., 2003).

In Logboayo and Soubré, inoculation has never been practiced, population densities of bacteria strains were probably weak and insufficient to achieve optimal symbiotic fixation with the varieties tested. Therefore, inoculation with efficient strains of bacteria must be necessary to optimize the yields of the new varieties.

Conclusion

The results of this study showed that inoculating the seeds contributed to the improvement of the performance of the different soybean varieties in both sites. However, there is need to repeat this trial to confirm these first findings and to determine the best time in the year to plant soybean. Evaluation of other promising varieties for the Nawa region of Côte d'Ivoire should be considered in the future work

Conflict of Interest

The authors have not declared any conflict of interest.

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