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Boosting land restoration success in the Great Green Wall through the use of symbiotic microorganisms for propagated tree seedlings

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ABSTRACT

Several studies have clearly demonstrated the scientific and practical use of the symbiotics microorganisms for plants in earth ecosystems. The main goal of this study was to rehabilitate a degraded soil of the sahelian zone of Burkina Faso by using the rhizobia and mycorrhizal symbiosis through the inoculation technique. Native rhizobial strains were isolated from soil samples. These strains were then tested in laboratory and greenhouse conditions for their effects on the nodulation and growth of *Vachellia seyal*. At the end of these tests three promising strains were selected to form a complex that was used with or without arbuscular mycorrhizal fungus for the inoculation of plants produced in nursery or issued from direct seedling at the field. After 3, 12 and 14 months of cultivation, respectively, the growth parameters of plants such as the height and the collar diameter were measured. In addition, the field survival rate of plantations was evaluated. The results showed that inoculation has improved the growth and survival rate of *Vachellia seyal* plants in the field. The double inoculation was more effective than the single inoculation. With these promising results, we recommend inoculation of seedlings for a better success of restoration plantings in the Sahel.

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Keywords: Symbiosis, native strains, rehabilitate, inoculation, sahelian zone.

INTRODUCTION

Several studies have pointed out the fact that, due to climate change and anthropic pressure, the rate of depletion of plant cover is much higher than that of its regeneration (Ozer and Ozer, 2005; FAO, 2016). Severe and frequent droughts, as well as floods, contribute to the destruction of natural ecosystems (FAO, 2016). As a result of its

fragile economy, Africa is considered to be the most vulnerable region to the effects of climate change (FAO-CILSS, 2008). Indeed, many studies have shown a reduction in the vegetation cover in the Sahel region of Africa (Amogu, 2009).

During the last few years, the Sahelian population growth rate has increased significantly and this has contributed to

substantial environmental changes. This anthropic pressure has triggered a significant deforestation for energy, lumber and agricultural purposes. In addition, the expansion of livestock generates an overgrazing, preventing the regeneration of some shoots. All of this contributes to the degradation of the vegetation cover (Ozer and Ozer, 2005). As a result, the soil is exposed to water erosion, wind erosion, etc., inducing significant perturbations of its physical, chemical and biological properties (Requena et al., 2001; Duponnois et al., 2017). This can be aggravated by the development of desert-like areas that can spread and merge (Nahel, 2004). The absence of actions to prevent this phenomenon can lead to many problems such as food insecurity, poverty, environmental migration, etc. (Safriel et al., 2005).

As a result, many development programs have engaged to combat this phenomenon. Being considered as a problem of local development, the fight against desertification requires local solutions (Requier-Desjardins and Caron, 2005). The Africa's Great Green Wall initiative is one of such restoration and rural development programs (AUC/PA-GGW, 2012). It was launched since 2007 to combat the effects of climatic change and desertification and develop sustainable development pathways in this food-insecure region and its highly vulnerable populations and ecosystems. Led by the African Union, the initiative aimed at achieving a transformational change for millions of people by increasing resilience in the Sahara and the Sahel through an integrated landscape approach (Sacande and Berrahmouni, 2016; Sacande and Berrahmouni, 2018).

In Burkina Faso, battle against desertification have begun since the 1960s with various stakeholders involved to test or recommend mitigation techniques (Hien et al., 2004) including mechanical ("zaï", "half moons", etc), biological (the straw), farming (manure, compost) and agroforestry techniques (reforestation, assisted natural regeneration, grass mat, etc.). Globally, the application of these techniques has gained a

positive effect on the fight against desertification. However, all of them have limitations (Roose et al., 2017). Indeed, the large scale application of biological and farming techniques can be limited by the availability of the material used. For agroforestry techniques, the lack of water during the dry season is an obstacle to their success (Roose et al., 2017). Thus, the optimization of these techniques could be beneficial in the context of an effective and sustainable fight against desertification. In addition to their environmental and ecological interest, trees are important sources of income, food security and traditional pharmacopoeia for rural populations (Bonkougou, 2004). Therefore, the optimization of an agroforestry and agroecology technique such as land restoration and reforestation will be interest in the context of an integrated fight against desertification. Specifically, reforestation with permanent legumes could be appropriate.

Among these legumes, acacias from the arid and semi-arid regions of tropical and subtropical areas such as *Acacia seyal*, (known as *Vachellia seyal*), *Acacia senegal* (known as *Senegalia senegal*) etc. are pioneers for the reforestation of dry soils, with low fertility (Räsänen, 2002). The faculty of these legumes to grow in these conditions is related to its ability to develop a symbiotic association with arbuscular mycorrhizal fungi (AMF) and bacteria of the group of rhizobia which are atmospheric nitrogen-fixing bacteria (Graham and Vance, 2003; da Silva et al., 2014).

Several studies have clearly demonstrated the scientific and practical use of this symbiosis for plants, whether in the natural or man-made ecosystems (Fortin et al., 2008). Indeed, it has been shown that AMF allow the mobilization of water and minerals (especially phosphorus) beneficial for plants (Lambers et al., 2008). In addition, the symbiotic nitrogen-fixing bacteria convert the atmospheric nitrogen (N₂) into mineral forms (NH₃) that can be assimilated by the plants (Matiru and Dakora, 2004). These abilities can be used to recover degraded lands, as it is

not only a cheap technology, but also, an environmentally friendly. In order to increase the chances of success of such a technique, degraded soils, which are poor in symbiotic microorganisms, must be supplemented with nitrogen fixing bacteria. However, phosphorus deficiencies in tropical soils can limit the establishment of the nitrogen-fixing symbiosis. Hence, AMF, known for their ability to improve phosphate nutrition of plants, could be used in association with nitrogen-fixing bacteria to provide the phosphorus necessary for their symbiosis with plants (Temagne et al., 2017). The inoculation technique is not commonly used although it would be a great added value and a natural boost to the success of large scale restoration program such as the Africa's Great Green Wall. To our knowledge this is the first work of this type at scale in the field in Burkina Faso and even in the whole GGW restoration program.

The objective of this study is to increase success of restoration and rehabilitation of a degraded soils area of the sahelian region of Burkina Faso as part of the Great Green Wall, by using rhizobial and mycorrhizal symbiosis through inoculation technique. *Vachellia seyal* (Del) has been chosen as a model native species because it is planted a lot for its multi-purpose uses including its production of commercial gums.

MATERIALS AND METHODS

Plant material

The seeds of *Vachellia seyal* and *Senegalia senegal* provided by the "Centre National de Semences Forestières (CNSF)" of Burkina Faso, were used.

Microbial material

Rhizobial strains isolated from soil in the sahelian zone of Burkina Faso were used as native strains and rhizobial strains ORS 3574, 3588 and 3607 (isolated from *Senegalia senegal* nodules) provided by the "Laboratoire Commun de Microbiologie de Dakar" were used as reference strains.

A strain of *Rizophagus irregularis* (Ri) was used as a mycorrhizal inoculant. The

strain was developed using a corn culture for 6 months on a sand. This inoculant consisted of fragments of mycorrhizal roots and spores.

Soil material

The soil used for the *Rizophagus irregularis* strain development was issued from Dori. For the production of plants in greenhouse a sandy soil used provide of Kaya. The nursery substrate used for plants production in nursery provide of Ouagadougou. The principal characteristics of these soils are presented on Table 1.

Mycorrhizal status of *Vachellia seyal* with the strain of AMF

The mycorrhizal status of *Vachellia seyal* associated to *Rizophagus irregularis* inoculant used was evaluated as follow :

Vachellia seyal seeds were planted in 10 plastics pots of 1.5 L containing 2 kg of a sterile sandy soil. The pots were inoculated with 10 g of *Rizophagus irregularis* inoculant. After 3 months in greenhouse, the fine roots were collected for the assessment of mycorrhization according to the method of Trouvelot et al., 1986.

Isolation of indigenous rhizobial strains

The indigenous or native rhizobial strains were isolated from host plants nodules grown in laboratory conditions as described below.

Soil sampling

Soil samples were collected in the locality of Bani (14°0.3936': N, 1°42.4686': W, 311 m A) located about 15 km from Djibo, in the Soum province. The soil was mined to a depth of 30 cm in the rhizosphere of legumes tree in October 2016. Totally, ten (10) samples were collected. These samples were passed a 2-mm sieve, respectively. They were then mixed to get a balanced composite sample for the host plants growth.

Host plants cultivation

The composite soil sample was used for the cultivation of two legumes, *Senegalia senegal* and *Vachellia seyal* as host plants in

Gibson tubes (Gibson, 1980). The seeds of these plants were scarified with sulfuric acid 96 ° and pre-germinated in Petri dishes containing 8% agar medium (w/v) at 28 °C for 72 hours. The plants were transplanted into Gibson tubes containing a sterile Jensen nitrogen-free medium (Vincent, 1970), and then incubated under wet atmospheric conditions for 48 hours. For each plant species, 5 repetitions were performed. The test was conducted in the laboratory at ambient temperature, with intermittent lighting (16 hours on day time and 8 hours at night). After four (4) days of growth, the young plants were then inoculated with 1 ml of the composite soil sample suspension (10 g of soil in 90 ml of physiological water). After two (2) months of growth, the nodules on the roots were collected.

Isolation of rhizobia from the nodules

The isolation of rhizobia was carried out in sterile conditions according to the technique of Weaver and Frederick (1982). Each individual colony obtained was purified by successive streaks on Yeast-Extract-Mannitol-Agar (YEMA) medium (Vincent, 1970). A total of about thirty isolates were obtained.

Infectivity test

The isolates obtained from the plants nodules can only belong to the rhizobia if they are able to renodulate these plants. For this, the infectivity (ability to form nodules on the host plant) of the isolated strains were tested. So, the plants seeds were pre-germinated and cultivated on the Jensen medium in Gibson tubes (Vincent, 1970) and then, inoculated with the different isolates (1 ml of bacterial culture per plant); 3 replications were made by isolate. The nodulation was followed, and the first twelve infectious strains were used to be tested in the greenhouse.

Selection of efficient strains

Scarification and surface sterilization of *Vachellia seyal* seeds were achieved by soaking in sulfuric acid (96 ° for 10 min). Then, they were cultivated in 1.5 L containers

containing 2 kg of sterilized sand (at 121 °C for 1 hour). After two weeks of cultivation, the young plants were inoculated with the native rhizobia isolates. Ten replications were performed per strain. Each plant was inoculated with 1 ml of bacterial culture (10^9 bacteria per ml). The test was conducted in a greenhouse at surrounding temperature. The experimental device was a totally randomized.

After 2 months of cultivation in the greenhouse, the number of nodules was counted and the collar diameter, plant height and the biomasses (shoot and root) were evaluated. At the end of this experiment, 3 strains were selected to form the complex of native rhizobia used for field test.

Field experimental tests

Study site

The study area was a degraded land located in Som (14° 3.5412 N, 1° 44. 0460 W, 297 m A), a village in the Soum province, at about 10 km from Djibo, in the Sahelian region of Burkina Faso. The rainfall distribution during the two years experiment period is reported in Table 2.

Preparation of the field

The experiment field (4.5 ha: 225/200 m) was plowed by the Delphino plow and then to set up the test. The field was divided into two parts of 2.25 ha (225/100 m). Each part has been delimited in microplots of 0,125 ha (50 m/25 m).

Soil samples were collected before the tests were put in place and their physicochemical characteristics determined for an indication of the overall state (Table 3).

Experimental design

The experimental design consisted of 2 blocks with two factors: direct seedlings and plantations. In each block, completely randomized a total of six (6) treatments were carried out triplicate and each replication was put on a parcel unit. The different treatments were as follow:

- *Vachellia seyal* + native rhizobial strains complex: (RB);

- *Vachellia seyal* + reference rhizobial strains complex: (RS);
- *Vachellia seyal* + the arbuscular mycorrhizal fungus: (CMA);
- *Vachellia seyal* + native rhizobial strains complex + arbuscular mycorrhizal fungus: (CMA + RB);
- *Vachellia seyal* + reference rhizobial strains complex + arbuscular mycorrhizal fungus: (CMA +RS);
- *Vachellia seyal* control: (T)

Direct sowing

Field sowing was carried out in August 2017 with scarified seeds of *Vachellia seyal*. The inoculation with the rhizobia was carried out by coating the seeds as follow: the seeds were immersed into a mixture of bacteria (10⁹ cells/ml) and a sugar (80%: w/v) solution (3/4; 1/4). The inoculation with the arbuscular mycorrhizal fungus was performed with 5 g of mycorrhizal inoculant per seed pocket. The seeding was carried out on lines (7 lines/microplot) made up of “half-moons” at the rate of 3 pockets per “half-moon”. After that, measurements (collar diameter and height) were performed on the plants at 3, 12 and 14 months of seeding. For each treatment a total of fifteen plants were measured.

Plantation

In order to perform the plantation, two steps were considered: the production of plants in nursery and the field plantation.

Production of plants in nursery

Vachellia plants were produced in nursery in May 2017. The crop substrate (non-

sterile) was mixed (1/3, v/v) with a sandy soil (2/3, v/v). *Vachellia seyal* seeds were pre-germinated and sown in plastic pots of 1,5 l containing 2 kg of soil. The inoculation with the arbuscular mycorrhizal fungus was carried out at the time of seeding at a rate of 10 g per pot. The inoculation with the rhizobial strains complexes was carried out after two weeks of culture, with 1 ml of rhizobium culture (10⁹ bacteria/ml) per pot. Three replications of fifty plants were carried out by treatment. After 3 months of culture, the growth parameters were measured. A total of thirty plants were measured for each treatment.

Plantation in field

After three months of culture the plants were transplanted to the field in August 2017 according to the above experimental procedure. For each treatment, a parcel unit was used per replication. The plantations were carried out on lines (7 lines/microplot).

3, 12 and 14 months after transplanting the growth parameters (collar diameter and height) were measured for at about thirty plants per treatment. In addition, the survival rate was evaluated at 14 months following equation : $SR = \frac{RP}{PP} \times 100$

With SR= Survival rate ; RP = number of plants surviving at the period t and PP = number of planted feet.

Statistical analysis

The data collected were analyzed with software R-3.5.1, 2018 ; variance analyses were performed and averages were compared with the Newman Kheuls’ test at the probability $p \leq 0.05$.

Table 1: Physicochemical characteristics of the soils that were used.

Characteristics physicochemical	Inoculum soil	nursery substrate	Sandy soil
Clays (%)	3.92	11.76	5.88
Silt (%)	1.96	15.69	1.96
Sand (%)	94.12	72.55	92.16
pHwater	5.89	7.78	6.08
O.M (%)	0.276	1.522	0.391
Total C (%)	0.160	0.883	0.227

Total N (%)	0.012	0.076	0.018
C/N	13	12	13
Total P (ppm)	126	691	314
P.ass (ppm)	6.03	3.13	2.9
Total K	661	813	559
K. dispo	36.04	35.06	29.22
Ca²⁺ (m²/100 g)	1.36	1.46	1.89
Mg²⁺ (m²/100 g)	1.62	0.63	1.43
K⁺ (m²/100 g)	0.11	0.07	0.11
Na⁺ (m²/100 g)	0.02	0.02	0.02

OM: organic matter, C: carbon, N: nitrogen, K: potassium, K dispo: potassium available, P: phosphorus, P.ass: assimilable phosphorus.

Table 2: Average rainfall of the study area during two years of experiment.

	Year 2017	January	February	March	April	May	June	July	August	September	October	November	December
Rainfall (mm)	0	0	0	0	71.2	105.2	98.3	84.7	114.7	11.5	0	0	
	Year 2018	January	February	March	April	May	June	July	August	September	October	November	December
Rainfall (mm)	0	0	0	0	30.6	83.60	127	216	132	24.2	0	0	

Table 3 : Soil physicochemical characteristics of experiment field.

Characteristics physicochemical	Plantation field soil	Seedling field soil
Clays (%)	15.69	15.69
Silt (%)	17.64	19.6
Sand (%)	66.67	64.71
pHwater	5.22	6.72
OM (%)	1.219	0.943
Total C (%)	0.707	0.547
Total N (%)	0.053	0.058
C/N	13	9
Total P (ppm)	377	377
P.ass (ppm)	1.69	1.56
Total K	966	915
K. dispo	39.93	23.45
Ca²⁺ (m²/100 g)	1.42	1.69
Mg²⁺ (m²/100 g)	0.42	0.82
K⁺ (m²/100 g)	0.17	0.07
Na⁺ (m²/100 g)	0.02	0.02

OM: organic matter, C: carbon, N: nitrogen, K: potassium, K dispo: available potassium, P: phosphorus, P.ass: assimilable phosphorus.

RESULTS

The mycorrhization test

The mycorrhization parameters observed were the mycorrhization frequency (F) and the intensity of mycorrhization (I). Our mycorrhization test revealed 100% for the frequency and 63.19% for the intensity.

Greenhouse tests for the selection of rhizobial strains

The results of statistical comparison of the effects of inoculation with rhizobia on the growth parameters under greenhouse conditions are summarized in Table 4. After 2 months of plants growth in the greenhouse, a significant difference was noticed between the inoculated and the non-inoculated plants (control) when the collar diameter was considered. However, there was no significant difference between the effects of the different strains of rhizobia on the collar diameter when the inoculated plants were compared with each other.

When the height was considered, only the inoculation with the strain AYJ2 showed a significant positive effect compared to the non-inoculated control. The difference with the other isolates was not significant compared to the control.

For the nodulation, all of the rhizobial strains induced the formation of nodules in the plants root, while the control was not nodulated. The AYJ5 strain induced a positive significant effect of nodulation compared to the strains AYJ1, AYJ4, AGJ1 and AGJ3.

Regarding the shoot biomass, only the inoculation with strains AYJ2 and AYJ3 resulted in positive significant difference compared to the non-inoculated plants. It also emerged that their effects on shoot biomass were significantly higher than those of strains AYJ4 and AGJ6. For the other isolates, the observed difference was not significant.

For root biomass, all of the rhizobia tested produced a significant effect compared to the control excepted AYJ1 and AGJ6. The highest values were obtained with strains AYJ3 (182.6 mg) followed by AGJ4 (169.5 mg) and AYJ2 (152.6 mg).

Field experimental tests

Direct sowing

In general, the inoculation stimulated the growth of plants from direct sowing in the field (Figure 1). Regarding collar diameter, at 3 months after seeding, the single inoculation (CMA, RB and RS) had showed a significant effect compared to the non-inoculated control (T). Dual inoculation (CMA+RB and CMA+RS) favored a better development of the collar diameter compared to the single inoculation (CMA, RB and RS) and the control (T), respectively. In dual inoculation the native rhizobial strains (CMA+RB) was more effective than that of the reference ones (CMA+RS).

For plant height, the double inoculation (CMA+RB and CMA+RS) significantly stimulated this parameter compared to the single inoculation (CMA, RB and RS). In the same way as the collar diameter, there was a significant difference between in dual inoculation with the native rhizobial strains (CMA+RB) and the reference ones (CMA+RS). However, no significant difference was observed between the single inoculation (CMA, RB and RS) and the control (T).

At 12 months, the collar diameter with the double inoculation (CMA+RB and CMA+RS) was significantly higher than the single inoculation one (CMA, RB and RS). The difference between double inoculation with the native rhizobial strains (CMA+RB) and the reference ones (CMA+RS) was also significant. There was a significant difference with the single inoculation (CMA, RB and RS) compared to the non-inoculated control (T). The inoculation with the rhizobial strains (RB and RS) showed a significant effect compared to the one with the arbuscular mycorrhizal fungus (CMA).

Regarding growth height, a non-significant difference was observed between the dual inoculation (CMA+ RB and CMA+RS) and the single inoculation with the rhizobial strains (RB and RS) ; however, this difference is significant when compared to the arbuscular mycorrhizal fungus (CMA) inoculation alone which had lower growth.

There was no significant difference between the native rhizobial strains (RB) and the reference ones (RS). Globally, the study showed a significant difference between the simple inoculation (CMA, RB and RS) and the non-inoculated control (T).

At 14 months, the collar diameter with the double inoculation (CMA+ RB and CMA+RS) was significantly superior with the single one (CMA, RB and RS). The effect produced was more significant with the native rhizobial strains (CMA+ RB) compared to the reference ones (CMA+RS). There was a significant difference between the single inoculation (CMA, RB and RS) compared to the non-inoculated control (T). The inoculation with the rhizobial strains (RB and RS) showed a significant effect compared to the arbuscular mycorrhizal fungus one (CMA).

As per the height, the difference was significant with the double inoculation (CMA+ RB and CMA+RS) compared to the single inoculation (CMA, RB and RS). There was also a significant difference in the single inoculation (CMA, RB and RS) compared to the non-inoculated control (T). The inoculation with the rhizobial strains (RB and RS) had a significant effect compared to the arbuscular mycorrhizal fungus one (CMA).

Plantation

Collar diameter and height

In general, the inoculation stimulated the growth of the plants (Figure 2). At 3 months in nursery, concerning the collar diameter, the inoculation induced a significant effect on the inoculated plants compared to those not inoculated. There was a significant difference between the native rhizobial strains (RB) compared to that of reference rhizobial one (RS) with single as double inoculation. Also, a significant effect of inoculation was noticed with the native rhizobial strains (RB) compared to the mycorrhizal arbuscular fungi (CMA).

For the height, only the double inoculation with the complex of native rhizobial strains (CMA+RB) which showed a significant effect compared to the single inoculation (CMA, RB and RS). The

inoculated plants have evolved significantly compared to the non-inoculated controls (T).

3 months after planting, the growth in the collar diameter was better in the inoculated plants than in the control. The double inoculation (CMA+RB and CMA+RS) was more effective than the single one (CMA, RB and RS).

No significant difference was registered in height.

12 months after planting, the inoculation induced a better development on the collar diameter of the inoculated plants compared to that of those not inoculated. The double inoculation (CMA+RB and CMA+RS) revealed a significant effect compared to the single inoculation (CMA, RB and RS).

Regarding the height, the double inoculation (CMA+RB and CMA+RS) had a significant effect compared to the single inoculation (CMA, RB and RS) and the control (T). On the contrary, the difference observed with the native rhizobial strains (RB) and the arbuscular mycorrhizal fungus (CMA) was not significant compared to the control (T).

At 14 months after planting, the inoculation has induced a better development of the collar diameter for the inoculated plants compared to those not inoculated. This effect was more marked with the double inoculation (CMA+RB and CMA+RS) compared to the single inoculation (CMA, RB and RS). With the single inoculation there was a significant difference between the rhizobial strain complexes (RB and RS) and the arbuscular mycorrhizal fungus.

About of the height, compared to the control, the inoculation stimulated growth in plant height. There was a significant difference between the double inoculation (CMA+RB and CMA+RS) and the single inoculation (CMA, RB and RS).

Field survival rate of plants

All treatments improved the field survival rate of plants, when compared to the non-treated ones. In general, field survival rate (Table 5) was more significant in double inoculated plants than in single inoculated ones.

Table 4: Effect of rhizobial strains inoculation on the growth of *Vachellia seyal* plants in greenhouse.

Rhizobial strain	<i>Vachellia seyal</i> growth parameter					
	Collar diameter (mm)	Height (cm)	Nodules number	Shoot biomass (mg)	Root biomass (mg)	
AYJ1	2.00 ± 0.55a	10.31 ± 2.06 ab	11.7 ± 5.72 b	174.3 ± 60.97 ab	115.4 ± 41.57bcd	
AYJ2	2.20 ± 0.33a	12.19 ± 0.88 a	20.6 ± 13.66 ab	216.1 ± 50.98 a	152.6 ± 36.03abc	
AYJ3	2.08 ± 0.30a	12.09 ± 3.40 ab	16.1 ± 9.12 ab	215.1 ± 62.17 a	182.6 ± 49.77a	
AYJ4	2.06 ± 0.47a	9.74 ± 2.35 ab	12.5 ± 11.6 b	140.0 ± 37.41 b	125.8 ± 27.3 bc	
AYJ5	2.06 ± 0.36a	9.35 ± 2.01 ab	27.3 ± 13.86 a	151.7 ± 37.03 ab	131.9 ± 51.06abc	
AYJ6	2.25 ± 0.31a	10.15 ± 1.14 ab	19.6 ± 10.69 ab	177.3 ± 28.41ab	123.9 ± 24.89bc	
AGJ1	2.07 ± 0.43a	10.29 ± 1.64 ab	12.3 ± 5.77 b	175.6 ± 38.83 ab	151.7 ± 36.53abc	
AGJ2	2.07 ± 0.39a	10.42 ± 2.40 ab	16.5 ± 5.72 ab	177.9 ± 61.88 ab	144 ± 32.76abc	
AGJ3	2.09 ± 0.17a	9.78 ± 1.79 ab	12.5 ± 7.92 b	164.3 ± 45.08 ab	128.2 ± 23.99bc	
AGJ4	2.41 ± 0.41a	9.23 ± 1.54 ab	18.4 ± 6.40 ab	177.9 ± 41.05 ab	169.5 ± 51.76ab	
AGJ5	2.05 ± 0.21a	11.12 ± 2.04 ab	21.7 ± 15.09 ab	167.5 ± 41.16 ab	136.5 ± 33.29abc	
AGJ6	2.25 ± 0.31a	8.90 ± 2.71 ab	18.9 ± 10.33ab	139.5 ± 60.94 b	111.1 ± 34.82cd	
Control	1.52 ± 0.28b	7.80 ± 3.69 b	0.0 c	117.4 ± 57.31 b	75.8 ± 40.42d	

In each column the values followed by the same letter are not significantly different according to the Newman Kheuls test (p ≤ 0.05).

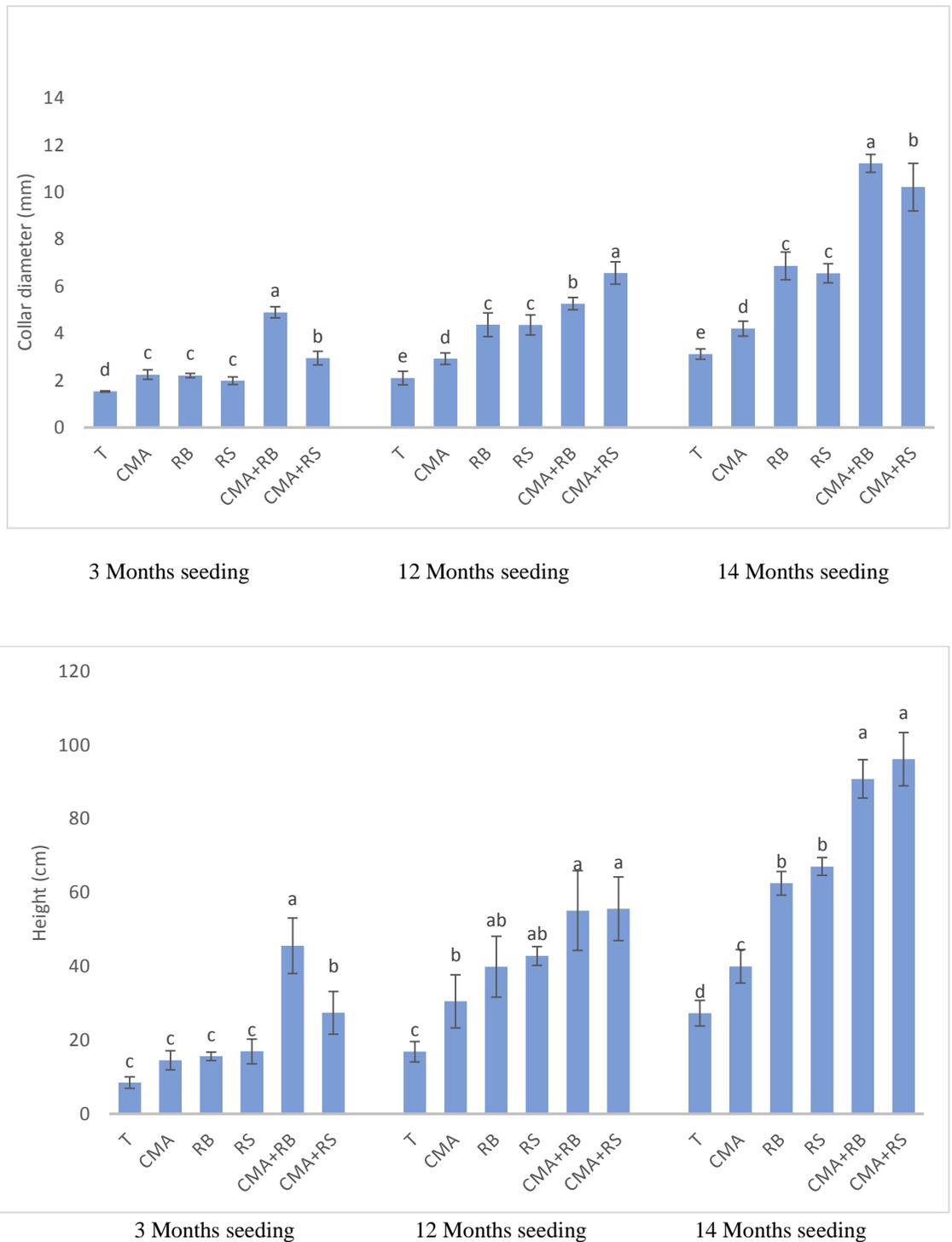


Figure 1: Effect of inoculation on the growth of *Vachellia seyal* direct seeding in field.

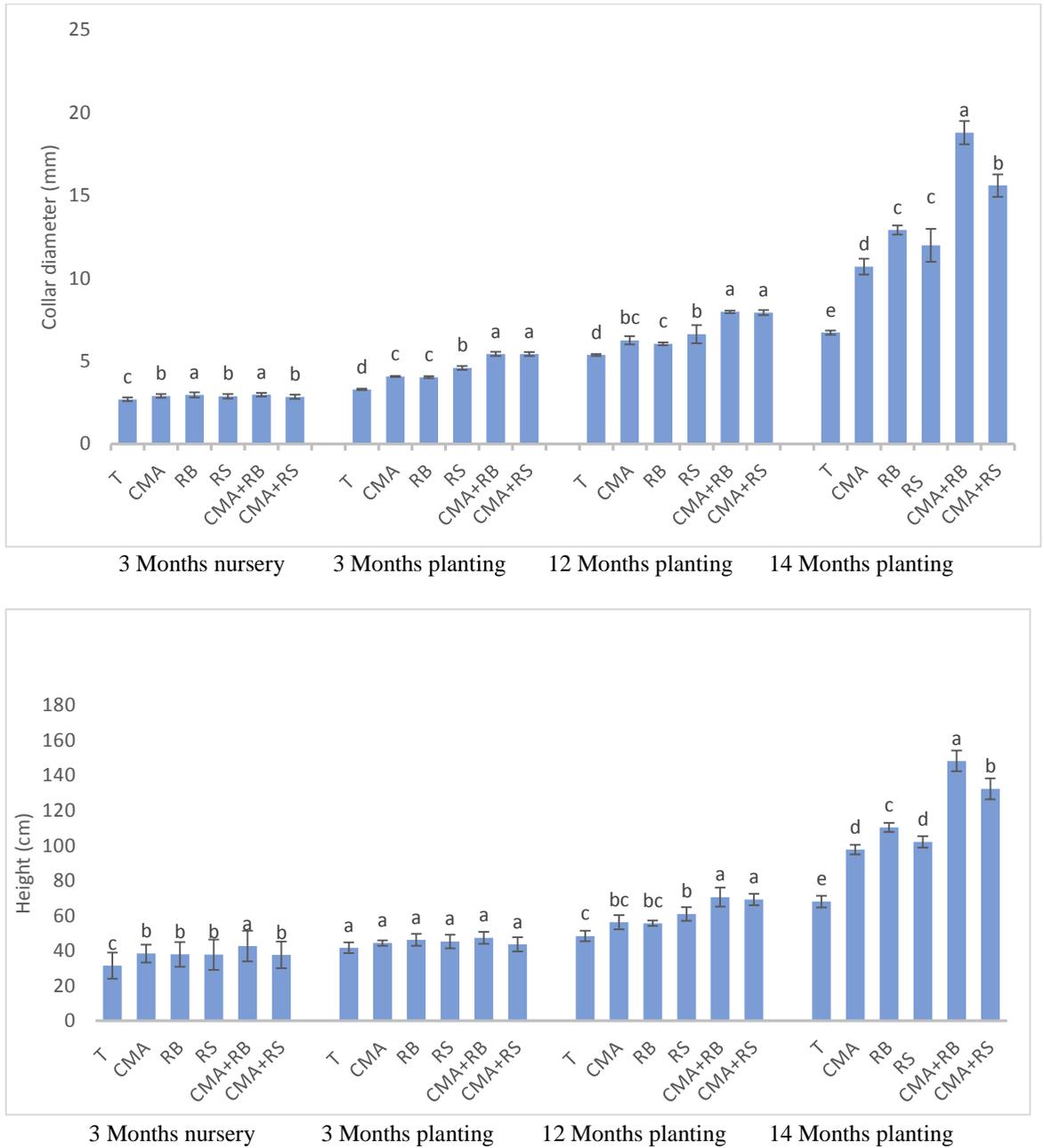


Figure 2: Effect of inoculation on the growth of *Vachellia seyal* plantations in field.

Table 5: Effect of inoculation on the survival rate of *Vachellia seyal* plants in field.

Treatment	Survival rate (%)
T	31.33
CMA	46
RB	54
RS	37.33
CMA+RB	57.33
CMA+RS	65.33

DISCUSSION

The mycorrhization test

The mycorrhization parameters observed were high. This indicated the compatibility between *Vachellia seyal* with the strain of *Rizopagus irregularis* used.

Selection of efficient strains in greenhouse

In our study, the number of nodules observed with the strains is relatively similar to that obtained by Backhoum et al. (2016) on *Vachellia seyal* inoculated with different strains of rhizobium. The production of nodules is an essential factor in the realization of a symbiotic relationship effective (N'Gbesso et al., 2017). Indeed, the nodulation stimulated the growth of plants by the fixation of nitrogen.

At the end of this experiment, three isolates (AYJ2, AYJ3 and AGJ4) were chosen to constitute the complex of native rhizobial strains inoculant due of their ability to improve the production of root biomass compared to others. In fact, plants produced in nursery that have a vigorous and strongly branched root system can bear the water stress they are confronted with after transplantation (Dianda et al., 2010).

Field experimental test

Direct seeding

The results show that inoculation has improved plant growth, showing the efficacy of the strains introduced in natural conditions. The positive effect of inoculation could be also explained by the fact that these microorganisms are adapted to the pedoclimatic conditions of the environment. In fact, some studies have shown that inoculation can only be effective when the microorganisms are adapted to the environment abiotic conditions (Meddad-Hamza et al., 2010). Our results contrast those of Diatta et al. (2013) who did not have a positive impact of inoculation on sesame in the natural environment due to presence of the native microflora. In our conditions of experiment where soil is degraded, there is

absence of native microorganisms (Duponnois et al., 2001; Azcon-Aguilar et al., 2003) which could compete with the introduced ones.

The double inoculation has been more effective on the height growth and on the development of the collar diameter. This could be explained by the synergistical action between the arbuscular mycorrhizal fungi and the rhizobia, evidenced by some studies (Sene et al., 2010; Aboubacar et al., 2013). Thus with this treatments mineral and water nutrition increased as a result of better development of plants.

As per the single inoculation, the rhizobial complexes were more effective than the arbuscular mycorrhizal fungus. This could be due to the fact that the mycorrhizae favorise plant growth in (i) a direct way by improving the mineral and water nutrition of the plants by the increase in the volume of soil prospected by the roots (mobilizing the nutrients in the deep horizons of the ground) and (ii) indirectly by improving the proliferation of other microorganisms that have the ability to solubilize minerals for the benefit of the plant (Requena et al., 2001; Barea et al., 2004). Under the conditions of our study, the soil being poor in microorganisms only the direct way of the arbuscular mycorrhizal fungus on the growth was performed. Furthermore, rhizobial complexes being more effective than the arbuscular mycorrhizal fungi could be related to the fact that in addition to fixing the atmospheric nitrogen, these strains of rhizobia are capable of phosphorus solubilization that can be assimilated by the plant (Peix et al., 2001).

Plantation

As with direct sowing, it was observed that inoculation induced a positive effect on the plants growth and that double inoculation being better than single inoculation. In addition, the field survival rate of plants was improved by the inoculation. Single inoculation with the arbuscular mycorrhizal fungus was found to be similar to that with rhizobial complexes. This could be explained

by the fact that: (i) the cultivation substrate, used in nursery not being sterile, should contain symbiotic microorganisms (mycorrhizae and rhizobia) compatible with *Vachellia seyal*.

(ii) In addition, it also contained non-symbiotic microorganisms (promoting growth plant rhizobacteria) involved in the major biogeochemical cycles of the N, P and C. These mechanisms allow a transformation of the chemical or organic elements into the nutrients dissolved in the soil solution that are then mobilizable by the plant to ensure its needs (Duponnois et al., 2013). So, the arbuscular mycorrhizal fungus will promote their proliferation and as a result the plant will benefit from their action. Indeed, it has been shown that the mycorrhizal symbiote exerts a selective pressure on the saprophytic microorganisms of the soil (Frey-Klett et al., 2005) in the goal of forming a trophic complex combining symbiote, mycorrhizospheric microflora and plant (Duponnois et al., 2017).

Conclusion

Our study showed that inoculation is beneficial to plant growth and the field survival of plants. The native rhizobial strains were efficient in natural conditions. The double inoculation was better than the simple inoculation. Transplanting plants produced better results than those issued from direct seeding. With these promising results, we recommend the wider use of inoculation of native microorganisms as a booster and benefit to the resilience and for a better success of restoration plantings in the dryland Sahel.

COMPETING INTERESTS

The authors declare that they have no competing interests.

AUTHORS' CONTRIBUTIONS

The authors, Barkissa FOFANA and Kadidia B. SANON, have conducted

experiments in laboratory, in greenhouse and at the field.

All authors have designed and approved the manuscript.

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