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RESEARCH ARTICLE

INFLUENCE OF GREEN MANURE FROM INOCULATED GLIRICIDIA ON RHIZOBACTERIAL POPULATION IN THE RHIZOSPHERE OF MAIZE

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ABSTRACT

The evolution of bacterial population size in the rhizosphere of maize through amendment with different organic materials from gliricidia plants was investigated. Greenhouse experiments were consecutively conducted for gliricidia biomass production and maize growth in Niayes zone soil. The plate count methods allowed estimation of rhizobacterial abundance in soil sampled from maize root systems. The density of two plant growth promoting rhizobacteria (PGPR) groups, mainly phosphate solubilizing bacteria (PSB) and fluorescent pseudomonads, was markedly affected by organic amendment. A positive rhizospheric effect of cultivated maize was observed on each rhizobacterial population investigated. Application of organic amendment allowed to get the highest PSB and pseudomonad abundance in maize rhizospheric soil which were estimated at log 5.76 and log 6.08 respectively. However, the nature of plant material used as green manure, did not influence neither abundance of PSB nor fluorescent pseudomonads in the rhizosphrere of maize.

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INTRODUCTION

Organic materials are used worldwide to improve soil properties and consequently crop production (Tester, 1990; Hoyley, 2013; Yanagi and Shindo, 2016). As soil amendment, they increase soil organic matters and play a significant role on supplying plant nutrients and maintaining soil productivity (Zhengfei, 2005; Zhao, 2009; Ives *et al.,* 2011; Mohammad *et al.,* 2012). In this way, use of organic materials improve sol structure and biological quality of agricultural sol. Among the divers soil microbial communities described in the rhizosphere of plants, rhizobacteria play a crucial role through their plant growth promoting properties. According to their diverse functional activities, the heterogeneous PGPR group act as biofertilizer, phytostimulator and phytoprotector (Beauchamp, 1993, Vasley, 2003; Somers *et al.,* 2004; Ahemad and Kibret, 2014). Large rhizobacterial groups including several species of agricultural interest are descripted at the genus level. So, pseudomonads and phosphate solubilizing microorganisms are largely detected in plant rhizosphere and thereby applied as inoculant in cropping systems (Compeau *et al.,* 1988; Vassilev *et al.,* 2006). Otherwise, the bacterial community related to plant root environment is affected by the presence and nature of organic matter that they use as source of energy and food (Juma, 1998; Han *et al.,* 2016). Fernando and Li (2011) reported that agricultural management systems based on organic farming affected relative abundance of bacterial communities. In the Sub-Saharan Africa in Senegal, the coastal area called Niayes zone is widely explored during the dry season mainly for small farmers' cash crop. Therefore, the use of inorganic fertilizers and pesticides in conventional agricultural systems contributes to soil and environment damage. To promote sustainable agriculture, organic materials are recycling to mitigate inorganic fertilizer use. In this way, various agroforestry systems are experienced in some smallholders' fields. Thus in a previous study, gliricidia (*Gliricidia sepium* (Jacq.) Walp) green manure applied for maize (Zea mays L) cultivation had greatly increased grain production (Diouf *et al.,* 2008). Grain increasing rate was highly significant when considered inoculation treatments for gliricidia cultivation to get a better quantity and quality of plant materials for amendment of maize crop. The objective of this study is to assess the impact of the organic material types on bacterial communities by investigating the abundance of both PSB and fluorescent pseudomonads in the rhizosphere of maize that received gliricidia green manure.

MATERIAL AND METHODS

Green manure Production: Gliricidiasepium culture was conducted in Bel Air Experimental Research Center at Dakar, into buried cylindrical pots containing 16 kg of homogenized sol with following characteristics: 7.0 pH, 93% sand, 0.025% N, 26 ppm available P. Seeds of gliricidia were surface scarified and sterilized for 15 min immersion in sulphuric acid and vigorously washed in sterile water. Treated seeds were pre-germinated into plate water agar at 30°C. Plant seedlings were then transferred into pots. Prior to get a high quality and quantity of gliricidia biomass, part of the plants were inoculated with rhizobial and arbuscular mycorrhizal strains (Diouf *et al.,* 2017). Plants were watered at field capacity with tap water for four month before harvested.

Maize cultivation: A greenhouse experiment was taken up for maize growth in similar containers and soil conditions as described above for gliricidia biomass production. Dried gliricidia plant shoots were crushed and incorporated into part of pots at the rate of 85 g/pot. Six weeks after green manure application, three maize seeds were shown in each pot allowed to maize growth. Three days after emergence, the seedlings were thinned down to one per pot and watered daily at field capacity. The experiment design was completely randomized with four replicates.

There were four treatments consisting of:

1. Maize grown with inoculated gliricidia plant shoot as green manure;
2. Maize grown with no-inoculated gliricidia plant shoot;
3. MAIZE cultivated without plant material;
4. Uncultivated control pot, without green manure and maize seedling.

At maturity, plants were harvested and rhizospheric soil samples recovered from carefully uprooted maize plants. For control treatment, soil samples were collected from the 0 – 20 cm topsoil.

PGPR population size: This study was focused on two rhizobacterial groups for their various and interesting PGP proprieties: fluorescent pseudomonads and phosphate solubilizing bacteria (PSB). For each treatment, soil samples collected were mixed, air dried (22°C) and then homogenized by sieving (2 mm). To determine rhizobacterial population size, 10 g of soil aseptically ground in a mortar were added to flask containing sterilized 90 ml of physiological saline solution (0.9% w/v NaCl). The soil suspension obtained was shake for 30 min at 150 rpm at 28°C. Tenfold dilutions series to 10^{-5} were made from 1 ml aliquots of the soil suspension. Three replicates were performed for each soil samples. For each soil suspension, the number of PSB was estimated using the plate count method. Aliquots (0.1 ml) from the serial dilutions were added to Pikovskaya medium in Petri plates (Pikovskaya, 1948) and incubated at 30°C for 5 days. Three repetitions were operated for each dilution. All bacterial colonies with halo were counted. The fluorescent pseudomonads number was determined in Petri plates containing King B medium (King *et al.,* 1954). Aliquots of 0.1 ml from serially diluted soil suspensions were plated onto a triplicate King B medium. Two to three days after incubation at 30°C, bacterial colonies that grown on the medium and emitted fluorescence under ultraviolet light were counted. Related to microbial culture density, the dilution with a suitable number of colonies is considered and expressed as log CFU (Colony Forming Units) per gram of soil. The data were subjected to statistical analysis by the STAT-ITCF software to evaluate the effect of different treatments. An analysis of variance indicating a significant difference between the factors was performed at $p \leq 0.05$. The data of the different parameters were compared according to the Newmann and Keuls test.

RESULTS

Determination of rhizospheric PSB of maize: The viable and cultivable phosphorus solubilizing bacteria were detected on Pikovskaya medium. The colonies observed 5 days after incubation were surrounded with a halo indicating a solubilizing activity of phosphorus. The number of bacteria with this property was determined by the plate counting method for the appropriate dilution of soil sample. Bacterial population sizes in the rhizosphere of maize differed according to soil sample analyzed (Table 1). The highest PSB population size was noted in rhizospheric soils amended with organic material. Whatever the organic matter treatment, the bacterial populations of rhizospheric soils of maize ranging from log 5.24 to log 5.76 CFU/g soil were significantly higher than those of control soil. However any significant effect of inoculation treatment applied for the production of gliricidia biomass used as green manure for maize growth was not observed on bacterial density in maize rhizospheric soil. The control soil consisting to the non-rhizospheric soil without green manure amendment and maize cultivation presented the lowest BSP population size (log 3.11 CFU/g soil). Therefore a soil rhizospheric effect of cultivated maize was noted onrhizobacterial population.

Determination of fluorescent pseudomonads in maize rhizospheric soil: Whitish bacterial colonies that appear on King B agar medium after incubation were considered. The appropriate dilutions of each soil sample were used to determine the number of cultivable bacteria. Analysis of fluorescent bacterial colonies counted under ultraviolet light revealed significant differences in soil sampled. Table 1 showed that investigated soil samples contained a very large population density of fluorescent pseudomonads. Data analysis indicated significant differences in fluorescent pseudomonad populations of soil samples. Rhizospheric soils amended with green manure were more prolific on fluorescent pseudomonads. Thus, application of gliricidia biomass highly improved the number of bacteria in the rhizosphere of maize compared to plants grown without organic amendment. In average, green manure application increased for 14.76% the

Table 1. Population size of phosphate solubilizing bacteria (PSB) and fluorescent pseudomonads (log CFU/g soil) in the rhizospheric soils of maize grown under different organic amendments of gliricidia

Sampledsoil	Abundance of PSB	Abundance of pseudomonads
Rhizospheric soil amended with biomass of inoculated gliricidia	5.76a	6.08 a
Rhizospheric soil amended with biomass of no-inoculated gliricidia	5.51 ab	5.91 a
Rhizospheric soil no-amended	5.24b	5.11b
No-rhizosphericsoil	3.11c	4.63 c
CV%	22.3	28.1

For each column, values followed by the same letter are not significantly different at $p \leq 0.05$

abundance of fluorescent pseudomonads in maize rhizosphere. However, inoculation for green manure production did not influenced significantly the density of fluorescent pseudomonads in the rhizospheric of maize. However, a rhizospheric effect of maize growth on pseudomonad populations was noted when compared cultivated and uncultivated soils.

DISCUSSION

Usually, organic amendments are applied in order to increase agricultural soil fertility and crop production. It has been long time noted that application of organic residues increased soil nutrient contents in part of Sub-Saharan soils (Bationio and Mokwunye, 1991). In Senegal, previous investigations on applied gliricidia green manure for maize growth showed a significant effect of the organic amendment on crop production (Diouf *et al.,* 2008). In this study, the type of biomass applied positively influenced maize grain production. Several works revealed that organic compounds from plant residues in soils, are subject to biological decomposition process and thereby contribute soil nutrients recycling through rhizobacterial action (Juma, 1998; Glick, 2012). The important plant growth proprieties of rhizobacteria related to their population size in rhizospheric soil improve crop production. In this way, Ashrafuzzaman *et al.* (2009) reported that bacteria have to be established at sufficient population densities in plant rhizosphere to valuably express their functional PGP proprieties. Our study is focused on PSB and fluorescent pseudomonads which were found in high abundance in the rhizosphere of maize cultivated in sandy soil of Niayes zone. In both cases, a significant rhizospheric effect of maize was noted compared to control soil. Similar results of soil rhizospheric effect on bacterial population were earlier reported by Miller *et al.* (1989) in various many plants cultivated including the maize.

Furthermore, several authors have noted a very high proportion of PGPR in the rhizosphere of divers crops such as maize, rice and sorghum and their important role in the mineral nutrition of crops (Ponmurugan and Gopi, 2006; Rajapaksha and Senanayake, 2011). Earlier studies reported that stimulation and proliferation of bacteria in the rhizospheric soil was related to root exudates in association with various nutrients and growth factors for rhizobacteria (Miller *et al.,* 1989, Cunningham and Kuick, 1992). These authors' findings may explained the high population size of rhizobacteria in thizosphere of no-amended maize culture obtained in our experiment. The impact of organic material on soil rhizobacterial population has been already reported by many authors (Ives *et al.,* 2011, Fernando and Li, 2011). In the same way, our results shown that application of gliricidia plant materials induced a significantly effect on the abundance of rhizobacterial population in maize rhizosphere. Moreover, some authors have reported a high quality of nutrient contents and level of decomposition of gliricidia biomass used for soil fertility regeneration (Mensah *et al.,* 2007; Hartemink and O'Sullivan, 2001). In the present study, organic management system based on different gliricidia biomass qualities influenced populations of bacteria. Han *et al.* (2016) reported that in plant rhizosphere, the quality of organic matter was as isive as soil rhizobacteria. For these authors, when an appropriate source of plant material is used, its physical and biochemical transformations lead to highly increase soil fertility. According to Ponmurugan and Gopi (2006), the variation in the number of rhizospheric bacteria could be attributed to several factors, including the composition of the organic matter introduced as an amendment. In addition, Pradasani *et al.* (2011) have counted a greatest number of bacterial colonies in soil sampled from organic amendment treatments which received the better quality of organic matter. However, in contrast to our study, any different significant difference was not observed on bacterial density according to the quality of gliricidia biomass type (i.e. produced with or without inoculation) with different N-contents used as amendment of maize crop (Diouf *et al.,* 2017). For both concerned rhizobacteria groups, soil population densities in the rhizosphere of maize were markedly affected by green manure application whatever the type of organic matter.

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