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Inoculation of *Pseudomonas putida* in Farmer Environment to Improve Growth and Yield: Maize (*Zea mays* L.) Trial in Sothern, Central and Northern (Benin)

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Authors' contributions

This work was carried out in collaboration among all authors. Authors MYA, NAA, OA, HS and ADK conducted the trials set-up, data collection and harvesting. Author MYA wrote the first draft of the manuscript and managed the bibliographical research. Author PAN performed the statistical analysis. Authors GCO, MA, NAA, AA and LBM wrote the protocol, managed the study analyses and supervised the various activities. All authors read and approved the final manuscript.

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ABSTRACT

The application of Plant Growth Promoting rhizobacteria as a microbial bio-fertilizers to increase soil fertility and productivity, allows a rational use of chemical fertilizers which makes agriculture sustainable. The aim of this study was to evaluate the effects of the rhizobacteria *Pseudomonas putida* on the maize productivity in farmer environment. For this purpose, trials were conducted in two Agricultural Development Poles with nine (09) producers equitably distributed in Southern,

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Central and Northern Benin. The experimental design was a completely randomized block of three (03) treatments (T1: farmer practice; T2: P. putida + ½ recommended dose of NPK and Urea; T3: recommended dose of NPK and Urea) with three (03) repetitions. During sowing, two (2) maize seeds of the 2000 SYNEE-W variety were introduced into a pot and inoculated with 10 ml of bacterial suspensions of 10^8 CFU/ml concentration. The results showed that the best heights, stem diameters and leaf areas of maize plants were obtained with P. putida + ½ recommended dose of NPK and Urea with the nine (09) producers of the three (03) areas with respective increases of 14.76%; 18.08% and 26.56% compared to the farmer practice. In addition, the results related to yield parameters such as aerial biomass, underground biomass and maize grain yield were better improved with the P. putida + ½ recommended dose of NPK and Urea. The average rates of increase recorded were 42.70%, 38.96% and 77.69%, respectively, compared to farmer practice. In sum, this rhizobacteria can be used as the microbial bio-fertilizers to improve maize productivity in Benin.

Keywords: Pseudomonas putida; bio-fertilizer; soil fertility; maize (Zea mays L.); Benin.

1. INTRODUCTION

Maize (*Zea mays* L.) is the world's largest cereal crop [1]. It is a cereal of great food importance for many populations in West Africa, mainly in Benin where it constitutes the basis of the diet of the populations of the Southern and Central of the country [2]. Despite its importance for food security, its productivity varies alternately from one year to another in Benin. This is the result of several difficulties, including the decline in soil fertility and the high cost of mineral fertilizers [3]. Given the importance of maize, its production would be interesting and necessary to ensure food security and economic growth in Benin [4]. In order to increase yields, producers use large quantities of chemical inputs (pesticides and fertilizers). Indeed, the intensive use of mineral fertilizers leads to soil acidification leading to a decrease in soil fertility [5,6]. In addition to the proven adverse effects of these chemicals, they are currently considered as the main environmental pollutants that have led to the deterioration of soil biological properties and the accumulation of chemical residues in harvested agricultural products [7]. In view of all this, it is therefore necessary to develop an environmentally friendly fertilization system. The use of Plant Growth Promoting Rhizobacteria (PGPRs), particularly under adverse and stressful conditions, can confer many benefits to plants [8]. PGPRs are generally defined as microorganisms that can stimulate plant growth through several mechanisms. Direct mechanisms are associated with increased nutrient availability and include biological nitrogen fixation [9], phosphate solubilization [10], production of siderophors [11] and synthesis of plant growth hormones such as indole acetic acid (AIA), cytokinins or gibberellins [12,13,14]. Various indirect mechanisms such as induced systemic resistance, production of antimicrobial compounds and competition for nutrients and colonization sites by pathogens have been described [15]. The use of PGPRs has become a promising alternative to mitigate plant stress [16,17]. In order to increase maize productivity in Benin, several researchers have been working in greenhouses and research stations on PGPR isolated from the maize rhizosphere in Benin. Among these PGPR, *P. putida* is one of the best strains that have shown a significant improvement in maize productivity [18,19,20,21]. It is noted from work conducted in research stations that the innoculation of maize seeds with *P. putida* + ½ dose of NPK and Urea allows average maize yields equal to the yields obtained with the full dose of NPK and Urea [20,21]. In view of the best results obtained in controlled environments on the different soil types in Benin, it was necessary to evaluate the ability of *P. putida* to improve the productivity of maize in farmer environments in the Agricultural Development Poles (ADPs) of Benin.

2. MATERIALS AND METHODS

2.1 Study Area

The study was carried out at Research and Development (R&D) sites located in two (02) Agricultural Development Poles (4 and 5) in Benin (Fig. 1). The tests were installed respectively at PDA 5 in the village of Zouzouvou in the district of Djakotomè in Southern Benin; at PDA 4 in Miniffi in the district of Dassa-Zoumè in Central Benin and at Ouénou in the district of N'Dali in Northern Benin. Ferralitic soils dominate in South Benin, while in Central and North Benin, ferruginous soils predominate.

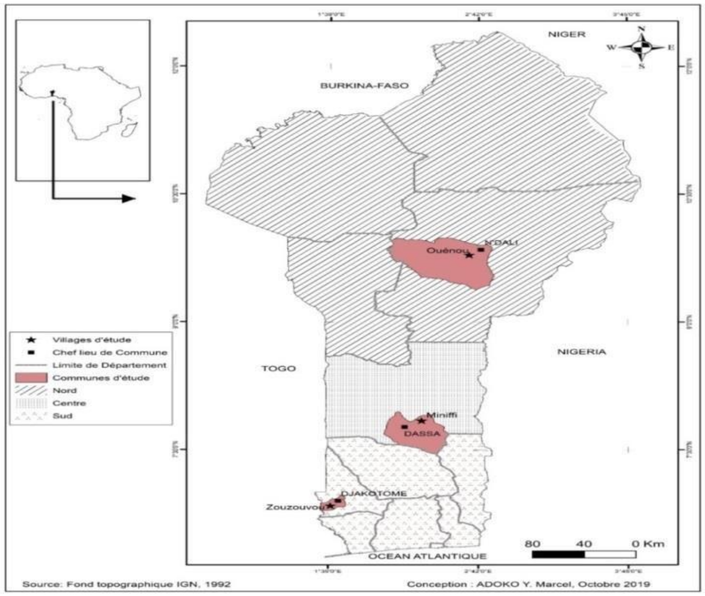

Fig. 1. Map showing the different study areas

2.2 Biological Material

The Maize seed of the 2000 SYNEE-W variety supplied by the Center Agricultural Research South (CRA-Sud) of the National Institute of Agricultural Research of Benin (INRAB) was used. It is an extra-early variety with an 80-day and a potential yield of 2.5 tonnes per hectare in a farmer environment. It was developed by the International Institute of Tropical Agriculture (IITA) and the National Institute of Agricultural Research of Benin (INRAB) [22]. The rhizobacteria *Pseudomonas putida* that was used was the one isolated and identified from the maize rhizosphere of the different agro-ecological zones of South Benin by [23] then preserved at -85°C in Mueller Hinton broth with added glycerol (10%) at the Laboratory of Biology and Molecular Typing in Microbiology (LBTMM) of the Faculty of Science and Technology (FAST) of the University of Abomey-Calavi (UAC).

2.3 Preparation of the Inoculum of *P. putida*

The *P. putida* strain was revivified by transplantation onto King B agar media for 24 hours at 30°C. The bacterial suspension was obtained by culture in nutrient medium (liquid MH) for 24 hours at 30°C. Then another culture was performed from the previous one. After 24 h incubation at 30°C, the cultures were then adjusted to a microbial concentration of approximately $1x10^8$ CFU/ml (OD 0.45 at 610 nm) with a spectrophotometer according to the method described by [24].

2.4 Experimental Device

The trial was installed at nine (09) producers in the three (03) study areas. At each producer the device was a randomized block of three (03) treatments with three (03) replicates. Each elementary plot was 12.8 m² in area and consisted of four (04) lines 4 m long with 0.80 m spacing. Sowing was done at a spacing of 0.80 m x 0.40 m a density of 31,250 plants/ha. The treatments are defined as follows: T1 = farmer practice (technique used by the producer); T2 = *P. putida* + ½ recommended dose of NPK and Urea; T3 = recommended dose of NPK and Urea.

2.5 Sowing and Inoculation

Two (2) maize grains were placed in a seed hole, which was approximately 5 cm deep. The seeds were then inoculated with 10 ml of bacterial suspension depending on the treatments. The pots are closed again following the bacterial inoculation. The doses of NPK of 200 kg/ha and Urea of 100 kg/ha were given according to each treatment.

2.6 Collection of Soil Samples

Soil sampling consisted of randomly setting five (05) sampling points on the diagonals of the experimental plot. These soil samples were taken prior to testing. Five (05) soil samples were taken using a Dutch auger. The soil samples were poured into a bucket and mixed into a composite. 500 grams of this mixture were collected, poured into a sterile plastic bag, labelled and sent to the Laboratory for determination of chemical properties.

2.7 Collection of Data on Growth Parameters

Data were collected on the two central lines of the useful plot (6.4 m^2). On these two lines, the height and crown diameter of the maize plants were measured respectively with tape measure and callipers on twelve (12) plants, every 15 days from the 15^{th} day after sowing until the 60^{th} day after sowing (JAS). Leaf area was estimated on the 60^{th} day after sowing by multiplying the length and width of the leaves by a coefficient of 0.75 [25].

2.8 Collection Data of Yield Parameters

Yield data for maize plants were collected at harvest on the 85^{th} JAS on twelve (12) maize plants. Aerial and underground biomasses of the 12 plants were harvested by treatment and by repetition on the two (02) centrallines of each elementary plot. They were then cut into small pieces and stored in a specially designed envelope. These biomass-filled envelopes were placed in an oven at 65°C for 72 hours until constant dry weight was obtained [26]. The dry biomasses were weighed using a scale (Highland HCB 302, Max: 3001 g) with an accuracy of 0.1 g. For grain yield, the ears of the twelve (12) maize plants previously harvested from the two (02) centrallines of each elementary plot were despatched and shelled. Using the moisture meter (LDS-1F), the moisture content was measured and the grains were weighed using a scale. The maize grain yield values were obtained using the formula:

$$\boldsymbol{R} = \frac{P \times 10.000}{S \times 1.000} \times \frac{14\%}{H} \quad [27]$$

Where: R is the maize yield, expressed in T/ha; P is the mass of maize per calculated elementary area, expressed in kg; S: is the useful area(S = 6.4 m2); H is the moisture content of the grain, expressed in %.

2.9 Statistical Analyses of the Data

The analyses were done with R 3.6.0 software (R Core Team, 2019) using the nlme, lsmeans packages. Linear mixed-effect models on longitudinal data were fitted to evaluate the effects of treatments and area on plant growth parameters. In each model, treatments and zones were considered as fixed factors and time as a random factor. Plant yield performance was evaluated using a two-criteria analysis of variance (treatment and area). The Ryan-Joiner and Levene tests [28] were carried out to verify the conditions of normality and homoscedasticity of the data required for anova production. The SNK test (post-hoc or multiple comparisons) was carried out in order to assess the statistical differences in the means of these parameters when the anova results are significant. The packages car, lsmeans and ggplot2 were used respectively for the anova, the calculation of the adjusted means and the graph editing.

3. RESULTS

3.1 Soil Chemistry of the Study Areas

The soil chemical properties of the three (03) study areas prior to the installation of the tests (Table 1) generally showed that the soils at the Ouénou (pH = 5.3) and Zouzouvou (pH = 5.6) sites are slightly acidic. These soils all showed a low level of fertility characterized by high C/N ratios for the topsoil layers (0-20 cm deep). On the other hand, in the Miniffi area in Central Benin, the pH (7.8) was alkaline in the topsoils with a medium level of fertility. These soils were richer in exchangeable K+ (2.2) compared to those of Ouénou (1.5) and Zouzouvou (0.9). The soil in Ouénou (5.5) had a low level of organic carbon compared to the soils in Miniffi (8.0) and Zouzouvou (8.8). However, exchangeable Ca^{2+} and Mg^{2+} were higher in Ouénou (9.4 and 5.4) compared to soils in other areas. In general, assimilable phosphorus was lower in Zouzouvou compared to soils in the other two sites.

3.2 Height of Maize Plants

Fig. 2 shows the effects of treatments and zone on maize plant height at the different zones. It can be noted that at the Zouzouvou site, the three treatments evaluated induced similar effects based on the evolutionary trend of maize plant height. Among the treatments studied at the Miniffi site, the full dose of NPK and Urea (T3) proved to be effective followed by *P. putida* + ½ dose of NPK and Urea (T2). The improvement gains induced by these treatments were respectively 25.21% and 21.11% compared to the control plants (T1). Analysis of the curves relating to the evolution of plant height over time in Ouénou shows that the best height of maize plants was obtained with plants treated with the full dose of NPK and Urea (T3), followed by those treated with *P. putida* + ½ dose of NPK and Urea (T2), which induced increases of 25.05% and 23.15% respectively compared to the farmer's practice (T1). Statistical tests carried out indicated that variations in maize plant height observed at plant level not only depended on treatments ($p < 0.001$) but also on area ($p < 0.001$). The *P. putida* + ½ treatment (T2) and the full rate of NPK and Urea (T3) had a significant effect over time on maize plant height (Fig. 3) compared to the field practice (T1). If the height curves for the three study areas are considered simultaneously, there is no significant difference ($p > 0.05$; p = 0.212) between the Ouénou and Miniffi areas on ferruginous soil. On the other hand, the zone effect was noticeable in Zouzouvou compared to the others ($p < 0.001$; p = 0.122).

3.3 Maize Plants Stem Diameter

Fig. 3 shows the effects of treatments and area on the crown diameter of maize plants in different areas. At the Zouzouvou site, the three (03) treatments have practically the same evolutionary trends on the crown diameter of maize plants. The results of the statistical analysis applied to the data indicated a non-significant effect of the treatments on this parameter. Nevertheless, the highest crown diameter values were recorded with *P. putida* + ½ dose of NPK and Urea and farming practice. At the Miniffi site, the curves of the evolution of plant crown diameter varied significantly both from one collection period to another and from one treatment to another. In fact, plants having received the full dose (100%) of NPK and Urea followed by plants treated with *P. putida* + ½ dose of NPK and Urea showed the best stem diameters with respective increases of 29.24% and 24.58% compared to farmer practice. However, no statistical difference ($p < 0.05$) existed between these two treatments. On the Ouénou site, the same trend was observed regarding the effect of *P. putida* + ½ treatments dose of NPK and Urea (T2) and full dose of NPK (T3). The latter induced the highest mean diameters compared to those of the control plants. At 60 days after sowing, the application of *P. putida* + ½ dose of NPK and Urea resulted in an improvement of 11.58% compared to the farmer practice (T1). Variations in diameter at the crown due to the effects of the zone are perceptible between the different zones. Indeed, a highly significant difference was observed between the stem diameter of plants in the Ouénou zone and those in the Miniffi zone ($p < 0.001$, p = 0.006). On the other hand, no significant difference was observed between the stem diameters of plants in the Zouzouvou zone and those in Miniffi ($p > 0.05$; p = 0.921).

Table 1. Soil chemical properties of the study areas prior to test installation

Sites	Villages	Depths (cm)	pH	C-org (g/Kg)	N-total (g/Kg)	C/N	P-Bray1 (mg/Kg)	B.E (cmol/kg) Ca^{2+}	Mg^{2+}	K^{+}
Dassa	Miniffi	0 – 20	7.8	8.0	0.6	13.3	47.5	33.3	2.3	2.2
N'Dali	Ouénou	0 – 20	5.3	5.5	0.6	9.3	30	9.4	5.4	1.5
Djakotomey	Zouzouvou	0 – 20	5.6	8.8	0.4	22	26.8	4.5	3.3	0.9

C-org: organic carbon; N-total: Azote total; P-Bray1: Phosphorus available; B.E: Base Exchangeable

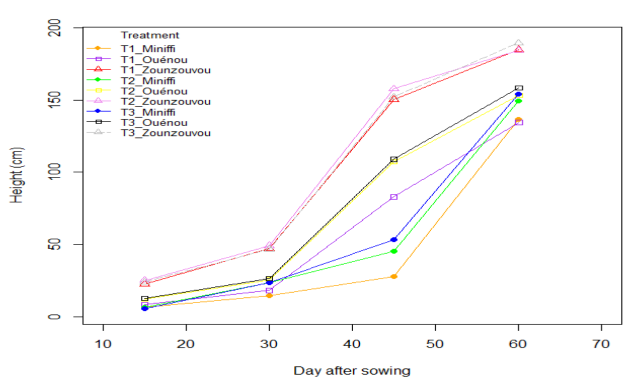

Fig. 2. Effects of treatments and area on maize plants height
T1 = Farmer practice; T2 = Pseudomonas putida + ½ dose of NPK and Urea; T3 = full dose of NPK and Urea

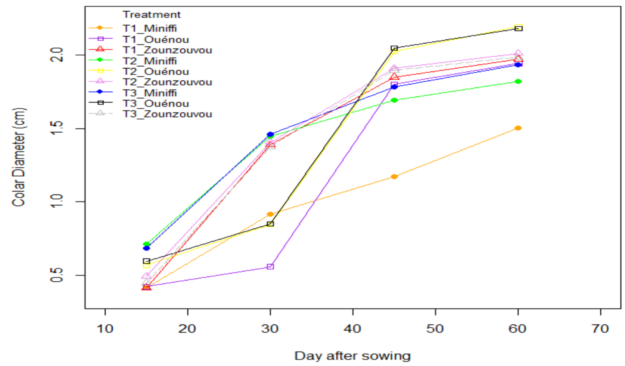

Fig. 3. Effects of treatments and area on stem diameter of maize plants

T1 = Farmer practice; T2 = Pseudomonas putida + ½ dose of NPK and Urea; T3 = full dose of NPK and Urea

3.4 Leaf Area of Maize Plants

The effectiveness of the inoculation was proven at the Zouzouvou site (ferralitic soil) and at the Miniffi and Ouénou sites (ferruginous soil for both) (Fig. 4). Indeed, the best leaf area of maize plants was recorded with the *P. putida* treatment in the presence of ½ dose of NPK and Urea (T2), i.e. respective increases of 18.22%, 35.14% and 26.31% at Zouzouvou, Miniffi and Ouénou compared to the farmers' practice (T1). The performance of the T2 treatment recorded in all zones slightly exceeded that induced by the full (100%) dose of NPK and Urea on the leaf surface of the plants. The results of the analysis of variance showed a highly significant difference in the effects of treatments and zone on plant leaf area ($p < 0.001$). It was noted that the treatment-area interaction was significant ($p < 0.001$) indicating that the resulting leaf area variations are treatment and area-dependent. Comparison tests (SNK) per treatment and per zone indicated that the *P. putida* + ½ treatments dose of NPK and Urea (T2) and the full dose of NPK and Urea (T3) induced a different statistical effect on leaf area than the practical farmer treatment (T1). Also a statistical difference was observed by area as shown in Fig. 4. The plants subjected to the practical farmer treatment gave the lowest leaf area values in all study areas. The largest leaf areas were recorded in Zouzouvou, while the lowest were recorded in Ouénou zone.

3.5 Aerial Biomass of Maize Plants

Fig. 5 shows the aerial biomass of maize plants as a function of treatment and area. In the Zouzouvou and Miniffi zones, the best aerial biomass of maize plants was obtained with the *P. putida* + ½ dose of NPK and Urea (T2) treatment, which had respective increases of 4% and 68.57% compared to the farmer practice (T1) and respective increases of 2.29% and 11.32% compared to the full dose of NPK and Urea (T3). On the other hand, at the Ouénou site, the best aerial biomass of maize plants was obtained with the full-dose treatments of NPK and Urea (T3) and *P. putida* + ½ dose of NPK and Urea (T2), which had increases of 64.44% and 55.55%, respectively, compared to farmer practice (T1). The results of statistical analysis revealed that there was a highly significant difference in the above-ground biomass of plants according to treatments and area ($p < 0.001$). The *P. putida* + ½ treatments of NPK and Urea and the full dose of NPK and Urea had statistically different results from the practical farmer treatment (Fig. 5). Plants in the Zouzouvou area provided large above-ground biomasses while the least important ones were observed in the Miniffi area.

3.6 Underground Biomass of Maize Plants

The underground biomass of maize plants as a function of treatment and area is shown in Fig. 6. The results of the analysis of variance showed a highly significant difference in treatment and area effects both in isolation and by interaction on the underground biomass of maize plants ($p < 0.001$). Indeed, the highest underground plant biomass productions were recorded with the application of the full dose of NPK and Urea (T3) and *P. putida* + ½ dose of NPK and Urea (T2) on ferralitic soil in Zouzouvou. The effect of PGPR was more remarkable on ferralitic soil. Indeed, both in Miniffi and Ouénou, the best underground biomass of maize plants was obtained with the *P. putida* + ½ treatment with NPK and Urea (T2) dose with respective increases of 82.53% and 32.6% compared to the farmer practice (T1). Control plants under the influence of the farmer practice treatment (T1) produced the lowest yields of underground biomass (Fig. 6).

3.7 Maize Grain Yield

Fig. 7 shows corn grain yield as a function of treatments in the different study areas. In all study areas, the best corn grain yields were obtained with plants treated with *P. putida* + ½ dose of NPK and Urea (T2) followed by those treated with the full dose of NPK and Urea (T3). However, statistical tests revealed that grain yield varied significantly from one zone to another and from one treatment to another with interaction of the two factors ($p < 0.001$). The advantage of inoculation significantly increased maize grain yield at the Zouzouvou, Miniffi and Ouénou sites by 19.28%, 168.64% and 48.34%, respectively, compared to farmer practice (T1). Moreover, the effectiveness of this treatment resulted in an improvement in grain yield of between 5.43% and 21.11% compared to the full dose of NPK and Urea (T3) in the three study areas.

4. DISCUSSION

Scientists have become aware of the need to reduce the use of chemical fertilizers and to adopt suitable production methods on soils that have become increasingly degraded in recent years. Several works have reported the effectiveness of rhizobacteria inoculation on crop growth and yield parameters under controlled conditions [29,20,19]. However, results recorded in the field remain less encouraging due to the influence of abiotic factors [30,31]. The aim of this study was to verify the capacity of the *P. putida* rhizobacterium to improve maize growth and yield in a farmer environment in Benin.

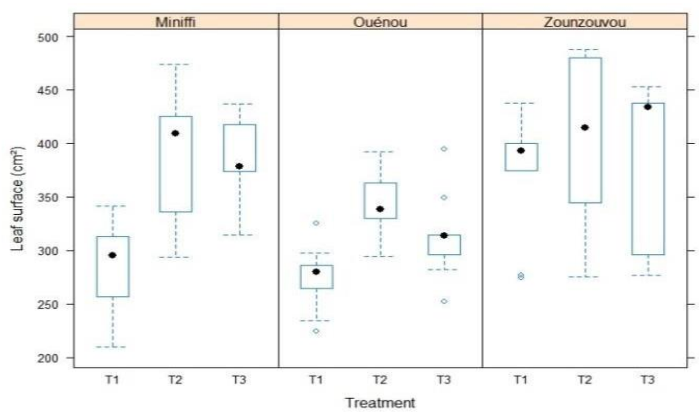

Fig. 4. Leaf area of maize plants according to treatment

T1 = Farmer practice; T2 = Pseudomonas putida + ½ dose of NPK and Urea; T3 = full dose of NPK and Urea

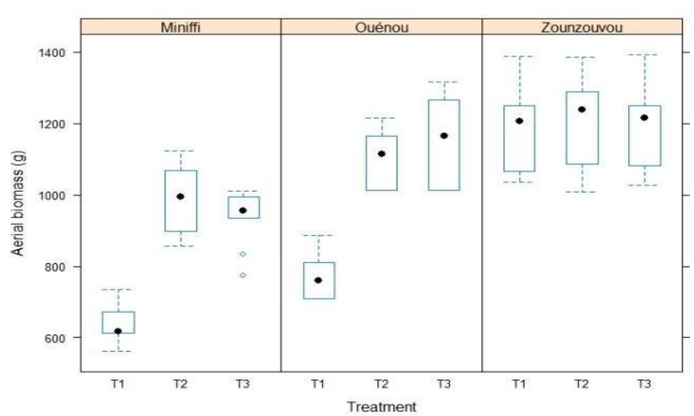

Fig. 5. Aerial biomass of maize plants according to treatment

T1 = Farmer practice; T2 = Pseudomonas putida + ½ dose of NPK and Urea; T3 = full dose of NPK and Urea

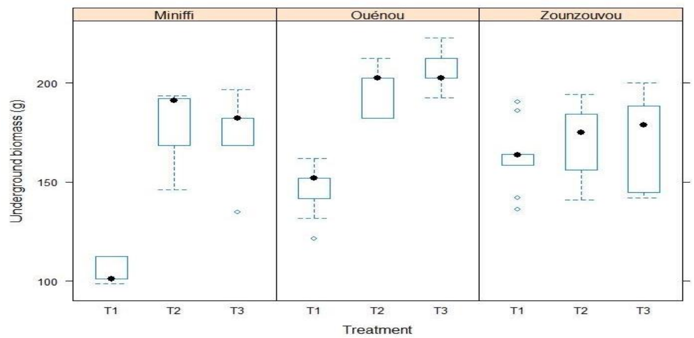

Fig. 6. Underground biomass of maize plants according to treatment
T1 = Farmer practice; T2 = Pseudomonas putida + ½ dose of NPK and Urea; T3 = full dose of NPK and Urea

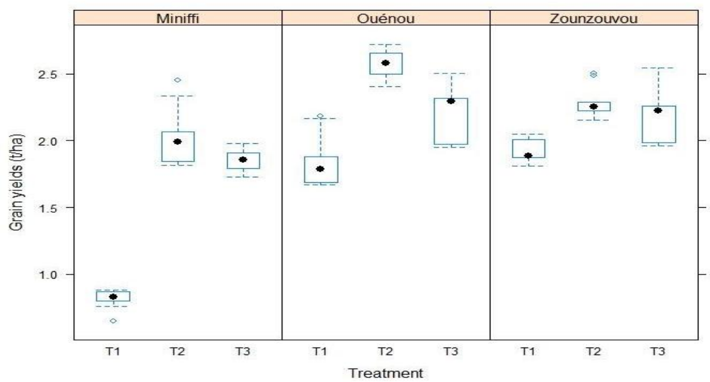

Fig. 7. Grain yield according to treatment
T1 = Farmer practice; T2 = Pseudomonas putida + ½ dose of NPK and Urea; T3 = full dose of NPK and Urea

The analysis of the chemical properties of the soils of the different sites reveals the low level of fertility (Table 1). The pH of the soils of Ouénou (5.3) and Zouzouvou (5.6) are slightly acidic while that of Miniffi is alkaline (7.8). The C/N ratio recorded in the topsoil at Zouzouvou (22) is high compared to those at the other sites (9.3 and 13.3). The phosphorus content is also low. In all three study areas, the potassium content of the soil is low in relation to calcium and magnesium. In general, soils in the study areas have low sum of exchangeable bases and low cation exchange capacity, reflecting their low fertility, as reported by [32]. The present study revealed the positive effect of the inoculation of *P. putida* + ½ dose of NPK and Urea on the growth and yield parameters of maize plants. This induced efficacy was variable not only by parameter but also by soil type. Indeed, on ferralitic soil in Southern Benin (Zouzouvou), the three treatments evaluated induced similar effects based on the evolutionary trend of maize plant height. The results of statistical analysis showed that there was no significant difference between the three treatments ($p > 0.5$). In Center Benin (Miniffi), the best maize plant heights were recorded by treatments T3 (full dose of NPK and Urea) and T2 (*P. putida* + ½ dose of NPK and Urea) with respective increases of 25.21% and 21.11% compared to farmers' practice. In Northern Benin (Ouénou), the best height of maize plants was obtained with the full dose of NPK and Urea (T3) followed by *P. putida* + ½ dose of NPK and Urea (T2) with respective increases of 25.05% and 23.15% compared to the Farmers' Practice (T1). These results are close to the 35, 24% recorded by [21] on maize plants treated with *P. putida* + ½ dose of NPK and Urea on ferruginous soil in Northern Benin. This recorded difference was related to the fertility gradients of the environments where the trials were carried out.

The different treatments have the same evolution on the maize plan stem diameter in Southern Benin. On the central and northern sites, the best maize plant crown diameters were obtained with *P. putida* + ½ dose of NPK and Urea, which exceeded farming practice by 18.08%. These results are close to the 25.24% obtained by [21] on maize plants treated with *P. putida* + ½ dose of NPK and Urea on ferruginous soil in Northern Benin. Similar results (26.12% increase) on maize plant height were reported by [33] with the inoculation of the rhizobacteria (Cn1) among the six (06) isolated from rhizospheric soils of two plants *Convolvulus arvensis* and *Polygonum plebeium* in Parkistan. These results also concur with those of [34] and [35], who demonstrated significant growths of maize plants with PGPR inoculation, particularly in height and diameter at the crown. [36,37] reported the beneficial effect of PGPR inoculation on plant growth. PGPRs have been reported to promote host plant growth through various mechanisms such as nitrogen (N2) fixation and solubilization of trace elements such as phosphate (P), [11,38]. With regard to the foliar surface area of the plants, in the study sites, the best values were recorded by the treatment *P. putida* + ½ dose of NPK and Urea with increases of 18.22% in the South; 35.14% in the Center and 26.31% in the North compared to the farmer practice. These results are in line with those of [20] who obtained a 27.29% increase with *P. putida* + chitosan + ½ dose of NPK and Urea compared to the control. The rhizobacteria *P. putida* under study is capable of solubilizing phosphate [39] and promoting a better absorption of nutrients by plants from the soil [18]; which would justify the results obtained with inoculated plants. Moreover, it has been reported that the larger the leaf area, the more plants achieve good photosynthesis, which is favourable to better productivity [40].

Several studies have demonstrated the effectiveness of joint application of PGPR and mineral fertilizers on soil fertility and crop productivity [41,42,43]. The use of PGPR as a bio-fertilizer in the presence of 80% of the recommended mineral fertilizer dose improved maize grain yield by 11.7% and biomass by 17.9% compared to the full recommended mineral fertilizer dose [44].

In this study, the PGPR significantly improved the yield parameters. At the southern site, the best above-ground biomass was obtained with plants inoculated with *P. putida* + ½ dose of NPK and Urea followed by plants treated with the full dose of NPK and Urea. However, there was no significant difference between the three treatments ($p > 0.5$). In the Center, the best above-ground biomass of maize plants was obtained with the treatment *P. putida* + ½ dose of NPK and Urea with an increase of 68.57% compared to the farmer practice. The effect of this treatment was also remarkable on ferruginous soil in the Northern, exceeding farmer practice by 55.55%. Recently, the work carried out by [21] showed that the PGPR *P. putida* + ½ dose of NPK and Urea was responsible for the increase of above-ground and below-ground biomass of maize plants by 71.45% and 66.66% respectively compared to the control on ferruginous soil in the Northern Benin. These observations of underground biomass are consistent with the results of the present study in the Center and North. Indeed, the application of *P. putida* + ½ dose of NPK and Urea induced the highest values of underground biomass with increases in the Center and Northern respectively of 82.53% and 36.6% compared to the farmer practice. [45] also obtained similar results in increasing the subsurface biomass of maize plants after bacterial treatment. Similarly, [33] found with PGPR Cn5, a significant increase (64.95%) in the underground biomass of maize plants compared to the control plant. The authors of this study justified the performance of this strain by the fact that it produces Hydrogen Cyanide (HCN) known for its protective effect by inhibiting the growth of pathogens. This increase in biomass could also be explained by the ability of PGPRs to produce siderophors, indole acetic acid and to solubilize phosphate [46,47].

As for maize grain yield, it was significantly improved by plants inoculated with *P. putida* + ½ dose of NPK and Urea regardless of the zone, with better maize grain yields than those grown according to farmers' practice (plants not inoculated). In the Southern Benin, the performance of *P. putida* + ½ dose of NPK and Urea on grain yield resulted in an improvement rate of 19.28% compared to the control (farmer's practice). In the past, [18] reported the efficacy (48.3%) of *P. putida* on maize yield in Niaouli at a research station in Southern Benin. The difference observed is thought to be related on the one hand to the variety of maize seeds used and on the other hand to the cultivation practices favoured by producers at the Zouzouvou site where the trials were conducted.

On the other hand, at the other sites in the Central and Northern Benin, *P. putida* + ½ dose of NPK and Urea induced more remarkable effects on maize grain yield. This parameter was increased more than eight times in the Center an increase of 168.64% compared to the farmer practice in the area. In the North, the yield (48.34%) of plants treated with *P. putida* + ½ dose of NPK and Urea was lower compared to that obtained in the Center. The work carried out by these authors, [48,39], reported that the PGPR rhizobacteria including *P. putida* used in this study strongly produce indole acetic acid (AIA), ammonia (NH3), hydrocyanic acid (HCN) and exopolysaccharides. The significant improvement in grain yield obtained would be related to the multifunctional properties of *P. putida*. [49] justify the improvement in crop productivity by the synergistic effects of rhizobacteria plant growth promoting traits. Studies by some authors also indicate that PGPRs confer to their hosts the ability to resist water stress and heat [50,51,52,53]. Similarly, some rhizobacteria increase the absorption of Zn, and thus the yield of several crops, including rice [54,55], wheat and soybean [56]. The results of our study clearly indicate that inoculation of *P. putida* with ½ dose of NPK and Urea significantly increased the growth and yield parameters of maize plants in Benin. Therefore, in Benin soils, this *P. putida* PGPR can be applied to increase maize productivity.

5. CONCLUSION

The study is being carried out as part of the evaluation of the performance of *P. putida* suspension in a farmer environment in Benin. The inoculation of maize plants with *P. putida* + ½ dose of NPK and Urea significantly improves the growth and yield of maize plants in Benin in a farming environment. Vegetative growth and yield parameters were therefore improved by *P. putida* + ½ dose of NPK and Urea. These results augur the possibility of using that *P. putida* as bio-fertilizer for sustainable maize production. In Benin, where the strong demographic pressure and the increasing decline in soil fertility are constantly jeopardizing agricultural sectors, alternative environmentally friendly agriculture, favouring the use of biological methods to increase harvests, is an unavoidable solution.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. N'Da HA, Akanvou L, Kouakou CK. Gestion locale de la diversité variétale du maïs (*Zea mays* L.) violet par les Tagouana au Centre-Nord de la Côte d'Ivoire. Int. J. Biol. Chem. Sci. 2013;7(5):2058-2068.
2. Adégbola PY, Aloukoutou A, Diallo B. Analyse de la compétitivité du maïs local au Bénin. PRESAO (Programme de Renforcement et Recherche sur la Sécurité Alimentaire en Afrique l'Ouest) Composante SRAI. Résumé N°1-2011-12–Maïs; 2011.
3. MAEP. Recueil des technologies agricoles prometteuses développées par le Système National de Recherche Agricole (SNRA) de 1996 à 2015. Document Technique et d'Informations. ISBN: 978-99919-2-985-9 Dépôt légal n° 9433 du 12 juin 2017. Bibliothèque Nationale du Bénin, 2ème trimestre. 2015;288.
4. Baco MN, Abdoulaye T, Sanogo D, Langyintuo A. Caractérisation des ménages producteurs de maïs en zone de savane sèche au Bénin. Rapport pays-Enquête-ménage-Benin; 2010.
5. Fayalo GD, Aouco A, Alavo TBC. Dynamique des populations du puceron *Aphis gossypii* (Homoptera: Aphididae) sur le cotonnier en conditions de fertilisation minérale et rhizobacteriologique. Tropicultura. 2016;34(1):98-104.
6. Houessou HG. Contribution à la lutte contre le puceron (*Aphis gossypii* G.) parasite d'Ocimum Gratissimum L. en régions urbaines au sud du Bénin. Mémoire de Master en Entomologie Appliquée. FAST/UAC. 2010;39.
7. Ahemad M, Kibret M. Mechanisms and applications of plant growth promoting rhizobacteria. Current Perspective JKSUS Journal. 2013;26(1):7-925. Reads.
DOI: 10.1016/j.jksus.2013.05.001.
8. Wani SH, Kumar V, Shriram V, Sah SK. Phytohormones and their metabolic engineering for abiotic stress tolerance in crop plants. Crop J. 2016;4:162–176.
DOI: 10.1016/j.cj.2016.01.010
9. Vessey JK. Plant growth promoting rhizobacteria as biofertilizers. Plant Soil. 2003;255:571-586.
10. Dahal K, Wang J, Martyn GD, Rahimy F, Vanlerberghe GC. Mitochondrial alternative oxidase maintains respiration and preserves photosynthetic capacity

during moderate drought in *Nicotiana tabacum*. Plant Physiology. 2014;166: 1560–1574.

11. Pii Y, Mimmo T, Tomasi N, Terzano R, Cesco S, Crecchio C. Microbial interactions in the rhizosphere: Beneficial influences of plant growth-promoting rhizobacteria on nutrient acquisition process. A review. Biol. Fertil. Soils. 2015;51:403–415.
DOI: 10.1007/s00374-015-0996-1
12. Calvo P, Nelson L, Kloepper JW. Agricultural uses of plant biostimulants. Plant Soil. 2014;383:3-41.
Available:https://doi.org/10.1007/s11104-014-2131-8
13. Gupta G, Parihar SS, Ahirwar NK, Snehi SK, Singh V. Plant growth promoting rhizobacteria (PGPR): Current and future prospects for development of sustainable agriculture. J. Microb. Biochem. Technol. 2015;7:096–102.
14. Kumar A, Bahadur I, Maurya B, Raghuwanshi R, Meena V, Singh D. Does a plant growth promoting rhizobacteria enhance agricultural sustainability. J. Pure Appl. Microbiol. 2015;9:715–724.
15. Kloepper JW, Ryu CM, Zhang S. Induced sycrownic resistance and promotion of plant growth by *Bacillus* spp. Phytopathology. 2004;94:1259-1266.
16. Fu Q, Liu C, Ding N, Lin Y, Guo B. Ameliorative effects of inoculation with the plant growth-promoting rhizobacterium Pseudomonas sp. DW1 on growth of eggplant (*Solanum melongena* L.) seedlings under salt stress. Agric. Water Manage. 2010;97:1994-2000.
17. Shilev S, Sancho ED, Benlloch-Gonzalez M. Rhizospheric bacteria alleviate saltproduced stress in sunflower. J. Environ. Manage; 2010. In Press
18. Adjanohoun A, Noumavo PA, Sikirou R, Allagbé M, Gotoechan-Hodonou H, Dossa KK, et al. Effets des rhizobactéries PGPR sur le rendement et les teneurs en macroéléments du maïs sur sol ferralitique non dégradé au Sud-Bénin. Int. J. Biol. Chem. Sci. 2012;6:279-288.
19. Noumavo AP, Kochoni E, Didagbé O, Adjanohoun A, Allagbé M, Sikirou R, et al. Effect of different plant growth promoting rhizobacteria on maize seed germination and seedling development. Am. J Plant Sci. 2013;4:1013-1021.
DOI: 10.4236/ajps.2013.45125
Available:http://www.scirp.org/journal/ajps
20. Agbodjato NA, Noumavo PA, Adjanohoun A, Dagbenonbakin G, Atta M, Rodriguez AF, et al. Response of maize (*Zea mays* L.) crop to biofertilization with plant growth promoting rhizobacteria and chitosan under field conditions. In JEBAS. 2015;3(6).
21. Amogou O, Dagbénonbakin G, Agbodjato AN, Noumavo AP, Salako KV, Adoko YM, et al. Applying rhizobacteria on maize cultivation in Northern Benin: Effect on growth and yield. AS. 2019;10:763-782.
Available:http://www.scirp.org/journal/asISSN Online: 2156-8561ISSN Print: 2156-8553
22. MAEP. Catalogue Béninois des Espèces et Variétés végétales (CaBEV), 4ème trimestre, INRAB/DPVPPAAO/ProCAD/MAEP & CORAF/WAAPP, Dépôt légal N° 8982 du 21 octobre 2016, Bibliothèque Nationale (BN) du Bénin. 2016;339.
23. Adjanohoun A, Baba-Moussa L, Glèlè kakaï R, Allagbé M, Yèhouénou B, Gotoechan-Hodonou H, et al. Caractérisation des rhizobactéries potentiellement promotrices de la croissance végétative du maïs dans différents agrosystèmes du Sud-Bénin. Int. J. Biol. Chem. Sci. 2011;5:433-444.
24. Govindappa M, Ravishankar RV, Lokesh S. Screening of *Pseudomonas fluorescens* isolates for biological control of *Macrophomina phaseolina* root-rot of safflower. Afr. J. Agricul. 2011;6:6256-6266.
DOI: 10.5897/AJAR10.1017
25. Ruget F, Bonhomme R, Chartier M. Estimation simple de la surface foliaire de plantes de maïs en croissance. Agronomie. 1996;16:553-562.
26. Yadav J, Verma JP, Tiwari KN. Effect of plant growth promoting rhizobacteria on seed germination and plant growth Chickpea (*Cicer arietinum* L.) under *in vitro* conditions. BFIJ. 2010;2:15-18.
27. Valdés EMF, González EC, Serrano MM, Labrada HR, Báez EM, Hernández FG, et al. Experiencias obtenidas en el desarrollo participativo de híbridos lineales simples de maíz en condiciónes de bajos insumos agrícolas. Cultivos Tropicales. 2013;34(2): 61-69.
28. Glèlè-Kakaï R, Sodjinou E, Fonton HN. Conditions d'Application des Méthodes Statistiques Paramétriques. BNB; 2006.

29. Akhtar N, Naveed M, Khalid M, Ahmad N, Rizwan M, Siddique S. Effect of bacterial consortia on growth and yield of maize grown in Fusarium infested soil. SE. 2018;37(1):35-44.
DOI: 10.25252/SE/18/872
30. Adesemoye AO, Torbert HA, Kloepper JW. Plant growth promoting rhizobacteria allow reduced application rates of chemical fertilizers. Microbial Ecol. 2009;58(4):921-929.
Available:https://doi.org/10.1007/s00248-009-9531-y
31. Kaschuk G, Le_elaar PA, Giller KE, Alberton O, Hungria M, Kuyper TW. Responses of legumes to rhizobia and arbuscular mycorrhizal fungi: A meta-analysis of potential photosynthate limitation of symbioses. Soil Biol. Biochem. 2010;42:125–127.
32. Balogoun I, Saïdou A, Ahoton LE, Adjanohoun A, Amadji GL, Ezui G, et al. Détermination des formules d'engrais minéraux et des périodes de semis pour une meilleure production du maïs (*Zea mays* L.) au Sud et au Centre Bénin. BRAB. 2013;1025-2355 et ISSN en ligne 1840-7099.
Available:http://www.slire.net
33. Shakeel S, Ahmed A, Javaid I. Study of the modulating interactions of multitrait rhizobacteria using *Zea mays* L. as the host plant. RADS J Biol Res Appl Sci. 2019;10(2):44-53.
34. Hartmann A, Bashan Y. Ecology and application of Azospirillum and other plant growth-promoting bacteria (PGPB). Special Issue Eur J Plant Pathol. 2009;45:1-2.
35. Pedraza RO, Bellone CH, Carrizo de Bellone S, Boa-Sorte PMF, Teixeira KRDS. Azospirillum inoculation and nitrogen fertilization effect on grain yield and on the diversity of endophytic bacteria in the phyllosphere of rice rainfed crop. Eur J Plant Pathol. 2009;45:36-43.
36. Balseiro-Romero M, Gkorezis P, Kidd PS, Van Hamme J, Weyens N, Monterroso C. Use of plant growth promoting bacterial strains to improve *Cytisus striatus* and *Lupinus luteus* development for potential application in phytoremediation. Sci Tot Envir. 2017;581:676-88.
37. Rizwan AS, Rifat H, Xiao-Xia Z, Nadeem AA, Safdar A, Mukhtar A, et al. Exploring potential soil bacteria for sustainable wheat (*Triticum aestivum* L.) production. Sustainability. 2019;11:3361.
38. Cakmakci R, Donmez F, Ayd A, Sahin F. Growth promoting of plants by plant growth-promoting rhizobacteria under greenhouse and two different field soil conditions. Soil Biol. Biochem. 2006;38: 1482-1487.
39. Noumavo PA, Agbodjato NA, Gachomo EW, Salami HA, Baba-Moussa F, Adjanohoun A, et al. Metabolic and bio-fungicidal properties of maize rhizobacteria for growth promotion and plant disease resistance. AJB. 2015;14: 811-819.
DOI: 10.5897/AJB2014.14132
40. Seema K, Mehta K, Singh N. Studies on the effect of plant growth promoting rhizobacteria (PGPR) on growth, physiological parameters, yield and fruit quality of strawberry cv. Chandler. 2018;7(2):383-7.
41. Bhardwaji D, Ansari M, Sahoo R, Tuteja N. Biofertilizers function as key player in sustainable agriculture by improving soil fertility, plant tolerance and crop productivity. Microb Cell Fact. 2014;13:66.
DOI: 10.1186/1475-2859-13-66
42. El-Basuaony, Asmaa A, Belal EBA, Atwa AAE. Effect of mineral and bio- fertilization on NPK availability, uptake and maize yield. J. Agric. Sci. Mansoura Univ. 2009;34(5):5795-5808.
43. Anjum MA, Sajjad MR, Akhtar N, Qureshi MA, Iqbal A, Jami AR, et al. Response of cotton to plant growth promoting rizobacteria (PGPR) inoculation under different levels of nitrogen. J. Agric. Res. 2007;45(2):135.
44. Sood G, Kaushal R, Chauhan A, Gupta S. Effect of conjoint application of indigenous PGPR and chemical fertilizers on productivity of maize (*Zea mays* L.) under mid hills of Himachal Pradesh. J Plant Nutr. 2018;41(3).
45. Zahid M, Abbasi MK, Hameed S, Rahim N. Isolation and identification of indigenous plant growth promoting rhizobacteria from Himalayan region of Kashmir and their effect on improving growth and nutrient contents of maize (*Zea mays* L.). Front Microbiol. 2015;6:207-217.
46. Agbodjato NA, Amogou O, Noumavo PA, Dagbenonbakin G, Hafiz AS, Kamirou R, et al. Biofertilising, plant-stimulating and biocontrol potentials of isolated PGPR rhizobacteria in Central and Northern Benin. AJMR. 2018;12:664-672.

Available:https://doi.org/10.5897/AJMR2018.8916

47. Souza RD, Ambrosini A, Passaglia LM. Plant growth promoting bacteria as inoculants in agricultural soils. Genet Mol Biol. 2015;38(4):401-419.
48. Rijavec T, Lapanje A. Hydrogen cyanide in the rhizosphere: Not suppressing plant pathogens, but rather regulating availability of phosphate. Front. Microbiol. 2016;7:1785.
 DOI: 10.3389/fmicb.2016.01785
49. Pérez-de-Luque A, Tille S, Johnson I, Pascual-Pardo D, Ton J, Cameron DD. The interactive effects of arbuscular mycorrhiza and plant growth-promoting rhizobacteria synergistically enhance host plant defences against pathogens. Sci Rep. 2017;7(1):16409-16419.
50. Abd El-Daim IA, Bejai S, Meijer J, Joahn M. Improved heat stress tolerance of wheat seedlings by bacterial seed treatment. Plant Soil. 2014;379:337-350.
 DOI: 10.1007/s11104-014-2063-3
51. Kassim WA, Omar MN, Abd El-Daim IA, Bejai S, Meijer J. Control of drought stress in weat usinig plant - growth-promoting bacteria. J. Plant Growth Regul. 2013;32:122-130.
52. Bresson J, Varoquaux F, Bontpart T, Touraine B, Vile D. The PGPR strain *Phyllobacterium brassicacearum* STM196 induces a reproductive delay and physiological changes that result in improved drought tolerance in Arabidopsis. New Phytologist. 2013;200(2):558-569.
53. Yang J, Kloepper JW, Ryu CM. Rhizosphere bacteria help plants tolerate abiotic stress. Trends Plant Sci. 2009;4:1-4.
54. Tariq M, Hameed S, Malik KA, Hafeez FY. Plant root associated bacteria for zinc mobilization in rice. Pak. J. Bot. 2007;39: 245–253.
55. Shakeel M, Rais A, Hassan MN, Hafeez FY. Root associated *Bacillus* sp. improves growth, yield and zinc translocation for basmati rice (*Oryza sativa*) varieties. Front. Microbiol. 2015;6:1286.
 DOI: 10.3389/fmicb.2015.01286
56. Ramesh A, Sharma SK, Sharma MP, Yadav N, Joshi OP. Inoculation of zinc solubilizing *Bacillus aryabhattai* strains for improved growth, mobilization and biofortification of zinc in soybean and wheat cultivated in Vertisols of Central India. Appl. Soil Ecol. 2014;73:87–96.
 DOI: 10.1016/j.apsoil.2013.08.009

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