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Phosphate solubilizing activity of native soil microorganisms from the rhizosphere of *Jatropha curcas* and from phosphate-solubilizing bacteria inoculum

E.B. Santana[1] and J.C.T. Dias[2]

[1] Departamento de Ciências Exatas e da Natureza, Universidade do Estado da Bahia, Alagoinhas, BA, Brasil
[2] Departamento de Ciências Biológicas da Universidade Estadual de Santa Cruz, Ilhéus, BA, Brasil

Corresponding author: E.B. Santana
E-mail: emanuelkantt@gmail.com

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ABSTRACT. Phosphorus is one of the most vital macronutrients required for growth and development of plants. A large number of microorganisms in the rhizosphere are known to solubilize and make available insoluble phosphorus, transforming it into phosphorus available to plants**.** We evaluated the phosphate solubilizing activity of native microbiota and phosphate solubilizing bacteria in rhizospheric soil with or without added rock dust (mainly granite dust) for enhancing growth of *Jatropha curcas*, an important plant for biodiesel production. The experiments were performed in a greenhouse with a random statistical design with 14 replicates. The soil received varying dosages of rock dust. Resident microorganism concentrations were measured, along with phosphorus content and enzymatic activity with focus on phosphatase, for 240 days. The highest content of phosphorus, 2.49, and dry biomass occurred in the presence of only soil-resident microbiota until 120 days, 70.45 in leaves; 73,98, in roots, and 105.44, in stalks. Soil samples under the influence of only resident microbiota had the highest enzymatic activity. The highest values were observed for acid phosphatase activity. Phosphatases showed values of 130.69 µg at 30 days, 155

µg, at 120 days, and 122.62 µg of p-nitrophenol.g^{-1}soil.h^{-1}, at 210 days. Added rock dust and phosphate solubilizing bacteria did not improve plant growth.

Key words: *Jatropha curcas*; Enzymatic activity; Phosphate-solubilizing bacteria; Rock dust

INTRODUCTION

The physic nut (*Jatropha curcas*) is native to Central America. It is suitable for agrofuel production in tropical and subtropical regions because it easily adapts to diverse soil and climatic conditions (including semi-arid conditions) and has low nutritional demands. It is considered a rustic crop and is adapted to most soil conditions, but it thrives in low natural fertility soils (Arruda et al., 2004; Dias et al., 2007; Freitas et al., 2011; Dias et al., 2012). Because it is not yet considered a domesticated plant, *J. curcas* is a subject of management and cultivation studies.

The crop yield of *J. curcas* may be enhanced employing microorganisms that improve plant growth, since they have demonstrated functional properties in the soil. Such microbes are designated “plant growth-promoting microorganisms” and constitute a heterogeneous group that may be found in the rhizosphere, in the surface soil, or in association with roots; they may improve the quality or extent of plant growth in a direct or indirect manner (Ahmad et al., 2008; Mohanty et al., 2013). Microbial strategies include the production of plant hormones, such as auxins, gibberellins, cytokinins, or polyamides (Tsavkelova et al., 2005), and participation in important transformations within the carbon (C) and nitrogen (N) cycle (Wallenstein and Burns, 2011). Another mechanism that may influence plant growth is the production of exopolysaccharides (EPSs) by bacteria associated with the plant’s radicular system. EPSs are extracellular polysaccharides produced by microorganisms such as fungi and bacteria that adhere to cellular surfaces or are externally excreted into the medium (Seesuriyachan et al., 2012). Production of EPSs arising from plant–microorganism interactions may lead to the formation of biofilms. Biofilms limit the diffusion of compounds secreted by roots and bacteria, allowing better adherence and colonization of surfaces where nutrients gather. They also contribute to nutrient and mineral fixation and water retention, and protect the plant against environmental stresses such as dehydration, salinity, temperature variation (Santos and Esposito, 2014), and pathogens (Qin et al., 2017). Nevertheless, plant growth-promoting bacteria may induce chemical and physical modifications in plants, resulting in increased tolerance to such stresses (Yang et al., 2009).

Plant growth-promoting bacteria that are associated with plant roots intensify such tolerance by stimulating radicular proliferation, i.e., enhanced production of lateral roots and root hairs (Paul and Lade, 2014), which improves water acquisition and nutrient absorption. Finally, microorganisms may also improve the nutritional status of the plant by solubilizing phosphorus (P).

Phosphorus is the most limiting nutrient in agricultural production, especially in tropical regions, due to its low availability within the soil (Khan et al., 2010), but this can be improved with the presence of Phosphate-Solubilizing Microrganisms. These microorganisms can supply the plant with phosphorus through the enzymatic activity and

production of organic acids (Mohanty et al., 2013). Phosphate-solubilizing microorganisms associated with a low-solubility phosphorous source, such as in natural rocks, has been successful in various crops, resulting in improvements in growth and production (Rodríguez and Fraga, 1999; Panhwaret al., 2011; Jha et al., 2012; Santana et al., 2017).

Natural rocks are milled to produce a dust, which provides a source of nutrients for plant cultivation, and may also adjust soil chemistry factors such as acidity (Lapido-Loureiro and Nascimento, 2009). Owing to the indiscriminate application and excessive use of phosphate fertilizers, which may have negative effects on agricultural sustainability and the safety of the soil and environment, rock dust constitutes a natural alternative for farmers; it ensures competitive production while preserving nutrient reserves in the soil. The nutrients in the rock dust are solubilized by the organic acids produced by soil microorganisms. Among these bio-solubilizing microorganisms, certain fungal genera (*Flavobacterium*, *Penicillium*, *Aspergillus*, and *Solerotium*) and bacterial genera (*Bacillus*, *Pseudomonas*, *Mycobacterium*, and *Micrococcus*) are noteworthy. Organic acids produced by the phosphate-solubilizing microorganisms fulfill an important role in the dissolution, transport, and concentration of elements on the Earth's surface, as well as in soil formation and plant nutrition, owing to the establishment of soluble complexes originated from minerals and rocks (Kpomblekou and Tabatabai, 2003). In addition to bio-solubilization by organic acids, these microorganisms may be responsible for the mineralization of phosphorus (Vassileva et al., 2010). By producing phosphatases, soil microbes hydrolyze various forms of organic phosphorus formed or stored in the soil, thereby releasing inorganic phosphorus that will be immobilized by plants and microorganisms. Intrinsic characteristics of the phosphate-solubilizing microorganisms within each type of soil determine the structural and functional equilibrium; these characteristics are physical (porosity, stability, and structure), chemical (pH and nutrient content), and biological (interactions with the native plant microbiota) (Grayston et al., 2004). Such factors determine the success or failure of microorganisms utilized to promote plant growth. Plants exert different effects on fungi (Broz et al., 2007) and bacteria (Marchante et al., 2008) in the soil microbiota that may reflect the profusion and/or diversity of the microorganisms. In the laboratory, phosphate-solubilizing bacteria can be selected in National Botanical Research Institute Phosphate (NBRIP) medium, supplemented with Ca_3PO_4. Phosphate-solubilizing bacteria can hydrolyze Ca_3PO_4.

We extended from the work of Santana et al. (2017), who examined the effects of bacteria and other microorganisms on *J. curcas*growth. Here, we evaluated the phosphorus content in leaves of *Jatropha curcas* inoculated with native microbiota and phosphate solubilizing bacteria in rhizospheric soil with or without rock dust and enzymatic microbial activity.

MATERIAL AND METHODS

Soil and rock dust

The soil used in the experiment was collected at a depth of up to 10 cm within a preservation area at the Experimental Unit of the Agrarian Development Company of Bahia (EBDA), located at coordinates 12°10′26″ South (S) and 38°26′15″ West (W). This soil was used because it is a typical soil used for planting this species in this area. The soil was

triple-sampled, conditioned in labeled bags, and characterized at the EBDA soil laboratory, in Salvador, Bahia, Brazil. Rock dust added to the soil was provided by the Sao Francisco Mining Company, located in São José's district, in the county of Feira de Santana, Bahia, Brazil, and was chemically characterized from triple samples at EBDA laboratory of soil. The rock dust used for substrate preparation included mining rejects, but the main part was comprised of granite dust. The granulometric analysis was carried out at the Civil Engineering Laboratory, CEPED, from the University of the State of Bahia (UNEB), Camaçari, Bahia, Brazil. The rock dust came from natural milled rocks.

Soil conditioning and fertilizer

Soil was sifted in a 5 mm mesh sieve before weighing (18 kg/pot) and fertilized with variable dosages of rock dust 1 month before planting, based on the volume of a truncated cone formula:

$$V = 1/3 \times \pi \times h\ (R^2 + Rr + r^2) \qquad \text{(Eq. 1)}$$

where R is the radius of the pot's base circle, r is the radius of the pot's top circle, and h is the pot height. Dosages of rock dust were added to the soil aliquots, which were organized on a resistant canvas and mixed using a shovel and rake. No other fertilizers were used, with the exception of urea 30 and 60 days after planting. The urea dosage for all pots corresponded to 40 kg/ha, with 1.17 g added to each pot.

Planting, irrigation, plant content of phosphorus

All seeds used in the experiment were provided by the Agrarian Development Company of Bahia (EBDA). From a total of 520 seeds disinfected with 3% sodium hypochlorite, half were inoculated in a sterilized cabinet with Phosphate-Solubilizing Bacteria at a concentration of 10^8 cells per mL, by means of dipping in sterilized water containing 0.1% xanthan gum for 10 min. The bacteria used for inoculation had previously been isolated, identified by sequencing, and selected in National Botanical Research Institute phosphate (NBRIP) medium containing 10 g/L glucose; 5 g/L $MgCl_2 \cdot 6H_2O$, 0.25 g/L $MgSO_4 \cdot 7H_2O$, 0.2 g/L KCl, and 0.1 g $(NH_4)_2SO_4$, supplemented with Ca_3PO_4 , to a final concentration of 1000 mg phosphorus per liter in solid medium and 100 mg phosphorus per liter in liquid medium (Nautiyal, 1999). Different values of initial pH were established: 5.0, 6.0, and 7.0. The inoculum comprised four Gram positive rhizosphere bacteria from the *Bacillus* genera: *Bacillus atrophaeus* MM20 (R2), *B. atrophaeus* SL44 (R11), *B. atrophaeus* SL13 (R35), and *Bacillus vallismortis* (S17), which were donated by the Environmental Monitoring Laboratory of the State University of Santa Cruz, Ilhéus, Bahia, Brazil.

In the course of sowing, three seeds were planted per pot at a depth of 5 cm, inserted with the caruncle uppermost. Thinning was performed 15 days after emergence, removing seedlings considered less vigorous and leaving only one plant per pot to ensure a uniform size of seedlings. Irrigation was performed daily in the pots when required to restore water consumed by evapotranspiration and to maintain soil humidity at approximately field capacity. To determine the phosphorus contents of the plants, triplicated samples with 200 mg of leaves were submitted to phosphorus extraction process, according

Miyazawa et al. (1999), performed at the Brazilian Agricultural Research Corporation - Embrapa semi-arid, in Petrolina, Pernambuco - Brazil.

Determination of the dry biomass: To evaluate the dry mass of leaves, stalks and roots, a destructive method was used; 210 days after sowing plants were harvested and the parts were separated to calculate the epigeal and hypogeal plant mass, described as follows:

a) Epigeal dry plant mass: - dry mass of stalks and leaves (g) - The aerial portion of the plant was pruned, separated (stalk and leaves) and conditioned in paper bags according to each treatment, before being set in an oven at 65^{o}C during 72 h. After being removed from the oven, the material was conditioned in hermetic plastic bags (in order to avoid moisture) and transported to the Plant Production Laboratory at the Forest garden from UEFS, where the material was weighted in a precision balance. Each treatment was triplicated and the values registered.

b) Hypogeal dry plant mass: - dry mass of roots (g) - roots were collected and washed under tap water before being dried in an oven at 65^{o}C during 72 h. Then, the material was conditioned in plastic bags and transported to the Plant Production Laboratory where the material was weighed in a precision balance. Each treatment was triplicated and the values registered.

Experimental design

The analysis of *J. curcas* growth was performed in a greenhouse. Ten treatments were established considering the soil collected and the dosages of rock dust. The treatments were set in a completely randomized design, with 14 replicates, being 5 dosages of rock dust. The rock dust dosages were 0, 13.5, 27, 54, and 81 g/pot. Plants for the analysis of only native microbiota (SWRWI) were characterized by the absence of rock dust; S135RWI to S81RWI had increasing dosages of rock dust, as described previously, without inoculation.

Statistical analysis

Data of plant height, biomass production, and nutrient content in the leaves were investigated using analysis of variance and Tukey's test ($P < 0.01$), with the aid of Biostat© software.

Microbial enzymatic activity

Enzymatic activity was determined by means of the evaluation of β-glucosidase, acid phosphatase, and arylsulphatase enzymes in the soil for 240 days. Soil samples were collected (0 - 10 cm) and sieved in a 4 mm mesh, removing all pieces of root tissues from plants and other organic matter, with the aid of tweezers, in order to avoid interference from these materials. Then, samples were stored at -20^{o}C until analysis. Evaluation of microbial enzymatic activity was performed until 210 days after sowing, using the methodology described by Tabatabai (1994) and Nautiyal (1999). Three replicates were used for analytical procedures for each soil sample. The enzymatic activity was expressed in µg p-Nitrophenol released per gram of dry soil per hour, by detecting the yellow color.

Acid phosphatase activity

The activity of acid phosphatase was determined by colorimetry, using p-Nitrophenyl phosphate as substrate and p-Nitrophenol as a product of the enzymatic reaction (Tabatabai, 1994). The experiment was performed in test tube containing 1 g soil (humid weight), after the addition of 0.2 mL toluene, 4 mL of MUB (pH 6.5), 1 mL of MUB p-Nitriphenol phosphate buffer, before test tubes were placed in vortex for few seconds. Flasks were closed with a latex stopper and Parafilm® before being incubated in water bath at 37°C for 1 h, followed by the addition of 1 mL $CaCl_2$ 0.5 mol/L and 4 mL NaOH, and stirring for some seconds before the soil suspension was filtered through a quantitative filter paper (Whatmann 2). The intensity of the yellow color in the filtrated was determined by colorimetry at 410 nm wave-length. The concentration of p-Nitrophenol was calculated from a graphic of the calibration curve resulting from 0, 10, 20, 30, 40 and 50 mg p-Nitrophenol standards.

Identification of the Native Microorganisms

DNA extraction, DGGE and Sequence Analysis to determine the profile of the soil microbial community were carried out according to Santana et al., (2017). Species of *Bacillus* from rhizosphere were identified: *B. subtilis*, *B. amyloliquefaciens*, *B. atropheus* and *B. vallismortis*.

RESULTS

Soil analysis

The soil used in the experiment was classified as a grayish sandy acrisol (Hapludalf), acidic, pH 5 (Table 1), with moderate content of organic matter and low phosphorus content, 2 mg/dm^3 (Table 2). The soil received rock dust rejects from a quarry where the main product was gravel. Chemical analysis of the rock dust revealed the presence of calcium (Ca), magnesium (MgO), potassium (K_2O), and phosphorus (P_2O_5). The rock dust had higher nutrient and pH values than the native soil, especially with regards to phosphorus. The content of phosphorus in the rock dust was of 247 mg/dm^3 and the pH was 6.6 (Table 3). Among the particles in the rock dust, 89.9% had a size under 0.15 mm (Table 4). Treatments are shown in Table 5.

Table 1. Physical characteristics of the soil.

Density (g/cm^3)		Granulometry (%)					
Apparent	Real	Clay	Silt	Sand	Humidity	MO	Classification
1.22	2.34	8.2	0.3	90.5	0.3	0.7	Sandy loam

Table 2. Chemical characteristics of the soil.

Soil depth (cm)	pH	MO (g/dm^3)	P (mg/dm^3)	K (mmol)	Ca	Mg	H+Al	Al	SB	T (%)
1–10	5.0	7.1	2	0.9	2.7	3	191	10	6.1	25
			Cu	Zn		Mn		Fe		
			-	(mg/dm^3)						
0–10			0.26	0.87		1.29		136		

Table 3. Chemical characteristics of the rock dust.

pH	MO (g/dm^3)	P (mg/dm^3)	K (mmol)	Ca	Mg	H+Al	Al	SB	T (%)
6.6	4.58	247	5.1	3.57	4	0.00	0.05	3.10	5.07
		Cu	Zn		Mn		Fe		
		-	(mg/dm^3)						
		0.58	0.9		1.4		158		

Table 4. Rock dust granulometry.

Sieves (mm)	Unfiltered dust granules (%)	Filtered dust granules (%)
1.2	25.1	74.9
0.6	18.8	81.2
0.3	10.9	81.1
0.15	10.1	89.9

Table 5. Treatments used in the cultivation of *Jatropha curcas*.

Treatment	Characteristics
SWRWI	Soil without rock dust, without inoculum
S135RWI	Soil with 13.5 g rock dust, without inoculum
S27RWI	Soil with 27 g rock dust, without inoculum
S54RWI	Soil with 54 g rock dust, without inoculum
S81RWI	Soil with 81 g rock dust, without inoculum
SWRI	Soil without rock dust, with inoculum
S135RI	Soil with 13.5 g rock dust, with inoculum
S27RI	Soil with 27 g rock dust, with inoculum
S54RI	Soil with 54 g rock dust, with inoculum
S81RI	Soil with 81 g rock dust, with inoculum

Dry biomass

Plant dry biomass production was higher in treatments under the influence of native microbiota only, with a significant statistical effect when compared with other treatments (Table 6). Plants from SWRWI produced averages of 70.54, 73.98, and 105.44 g of dry mass from leaves, roots, and stalks, respectively. These values were 17.33, 22.6, and 6.16% higher than in SWRI, the second treatment with the best dry biomass production, characterized by the presence of inoculum without rock dust.

Table 6. Dry mass from leaves, roots and stalks of *Jatropha curcas*, after an experimental period of 240 days, control treatment (SWRWI), treatments including rock dust, inoculated treatments and both.

Treatment	Mean weight of plant parts (g)		
	Leaves	**Roots**	**Stalks**
SWRWI	70.54 (0.27)a	73.98 (1.35)**a**	105.44 (2.27)a
S135RWI	40.78 (0.11)	46.87 (1.44)	62.11 (1.40)
S27RWI	50.87 (1.43)c	48.45 (1.38)	98.56 (1.42)b
S54RWI	41.05 (1.10)	55.29 (1.01)	79.67 (1.12)
S81RWI	50.29 (1.18)	55.99 (1.02)	92.28 (0.12)**c**
SWRI	60.12 (0.67)b	60.34 (0.10)b	99.61 (1.21)b
S135RI	35.87 (0.99)	32.4 (4.54)	35.09 (1.53)
S27RI	41.34 (0.89)	40.22 (1.70)	42.11 (0.42)
S54RI	41.29 (0.06)	55.17 (3.22)	79.08 (2.24)
S81RI	50.32 (1.00)	56.12 (1.98)**c**	92.20 (1.15)

Notation SWRWI-S81RWI refers to treatments with 0, 13.5, 27, 54 and 81 g of rock dust, without inoculation. Treatments SWRI–S81RI are referred to the same order in dosages of rock dust, but with inoculation. Means are followed by the standard deviation. Values followed by different letters in vertical rows are significant by Tukey's test ($P < 0.01$).

Phosphorus content in leaves of *Jatropha curcas*

The quantity of phosphorus in leaves increased until the end of the experimental period, excluding SWRWI, S135RWI and S27RWI (Table 7).

Table 7. Mean values of phosphorus content in leaves of *Jatropha curcas* at 30, 120 and 240 days.

Treatments	Phosphorus content (g/Kg)		
	Period (days)		
	30	**120**	**240**
SWRWI	2.05[a]	2.49	1.48
S135RWI	1.20	2.48	2.42
S27RWI	1.27	2.97	3.02
S54RWI	1.08	3.34[a]	4.75[a]
S81RWI	1.11	3.01	4.48
SWRI	1.05	3.33[a]	4.01
S135RI	1.23	3.15[b]	4.53[b]
S27RI	1.36	3.45[a]	3.38
S54RI	1.21	3.37[a]	4.87[a]
S81RI	1.41[b]	3.12[b]	4.80[a]

Notation SWRWI-S81RWI refers to treatments with 0; 13.5; 27; 54 and 81 g of rock dust, without inoculation. Treatments SWRI–S81RI are referred to the same order in dosages of rock dust, but with inoculation. Means are followed by the standard deviation. Values followed by different letters in vertical rows are significant by Tukey's test ($P < 0.01$).

Phosphorus gathering reached its top at 240 days of the experimental period, accomplishing a value of 4.87 g/Kg in S54RI. T he values of phosphorus gathering observed in SWRWI leaves were lower when compared to all treatments with rock dust and/or rock dust with inoculation, at 120 days, with a value of 2.49 g/Kg. Values observed for the gathering of P in SWRWI at 120 days of the experimental period, were of 1.55 g/Kg. There was an increase in the gathering of phosphorus in leaves until 120 days. In the

remaining treatments analyzed, the gathering of phosphorus in leaves was higher than in SWRWI only starting at 120 days.

Microbial enzymatic activity

In this experiment, low values were obtained after evaluating soil enzymatic activity. No significance was observed between the main effects and interactions among rock dust dosages and periods of evaluation for treatments with and without inoculation.

Figures 1 and 2 illustrate the behavior of phosphatase activity within treatments with and without inoculation. The activity of phosphatase was higher without inoculation, obtained at a point of 0 - 118 (dosage of rock dust - time of evaluation in days). When inoculated samples were analyzed, the maximum point of activity for phosphatase was obtained at a dosage 0 and 76 days. Phosphatase activity remained higher until 120 days. In treatments with inoculation, the activity of phosphatase increased at 120 days, stabilizing hence forward. The lack of overlapping confidence intervals indicated that differences in enzymatic activity between inoculated and non-inoculated treatments occurred along the whole evaluation period. The highest values were observed for the activity of the acid phosphatase. Phosphatases had values of 130.69 µg at 30 days, 155 µg, at 120 days, and 122.62 µg of p-nitrophenol.g^{-1}soil.h^{-1}, at 210 days (Figure 2).

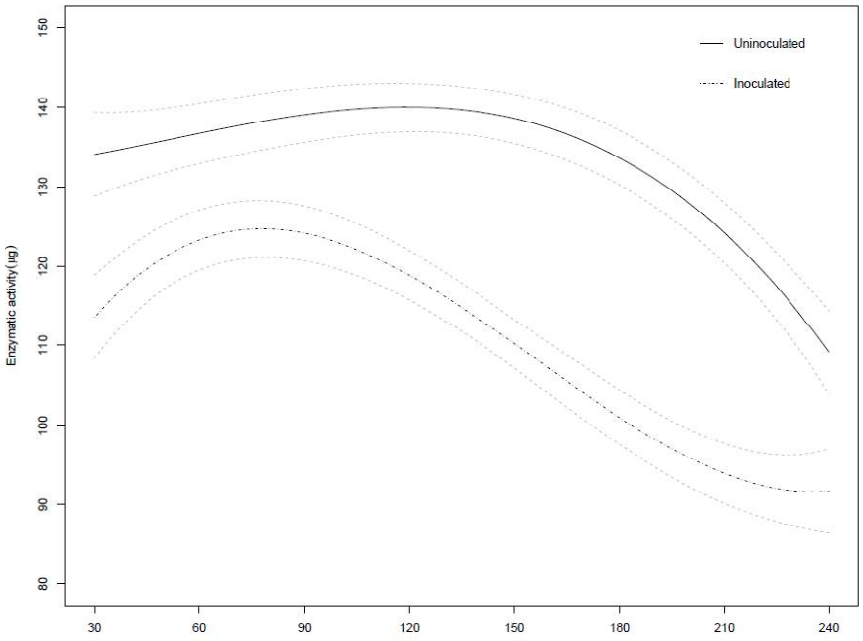

Figure 1. Activity of acid phosphatase with and without inoculation with PSBs.

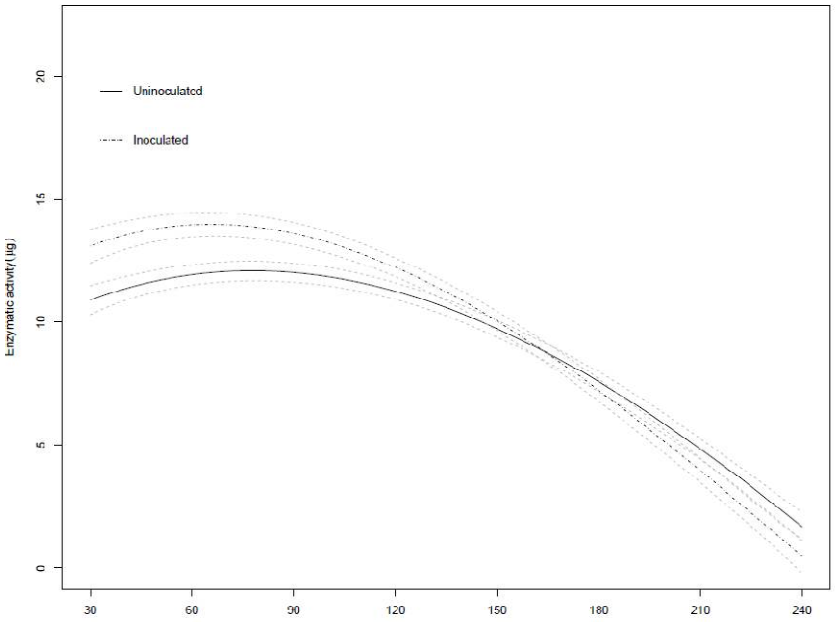

Figure 2. Activity of arylsulphatasewith and without inoculation with PSBs.

DISCUSSION

Rock dust analysis

The chemical analysis of the rock dust showed the presence of calcium (Ca), magnesium (MgO), potassium (K_2O), and phosphorus (P_2O_5); presumably Ca from calcite, Mg from biotite, K from feldspar, and P from apatite. Therefore, the rock dust used in the experiment constituted a source of nutrients. Granulometric analysis showed that the majority of rock dust particles had a diameter of 0.15 mm, which is an adequate size for soil fertilization purposes.

Dry biomass

Considering plant's dry biomass production, the results showed there was little synthesis of reserve compounds for storage in the stems and root structures, thus reflecting in the total weight of both plant organs in SWRWI plants. This revealed a higher physiological efficiency, ratifying plant characteristics, as those that are able to exploit a reduced nutrient supply for growth. The plant, along with soil microorganisms, may produce regulating hormones, such as cytokinins (Epstein and Bloom, 2006). These hormones move towards the growth spots in shoots turning these locals more adequate for the acquisition of nutrient reserves demanded for growth. The processes of absorption, re-distribution and allocation of P and possibly other nutrients, were satisfactory, and most likely reflected in the better values observed for biomass production. For other treatments, higher dosages of rock dust than 13.5 and 27 g, may have compromised nutrient absorption

by *J. curcas* roots during the initial periods of its development. Limitations of available P and other nutrients during the beginning of plant growth may result in restrictions in development, without the possibility of a later recovery even when increasing nutrients are available (Jeschke et al., 1996; Pádua, 2012). Phosphorus is one of the key mineral constituents for cellular activity; it is also the most limiting nutrient for biomass production and plant growth in tropical soils. The inadequate development of plants in some treatments, due to the previously mentioned problems, may have occurred in the beginning of the development period, showing difficulties in the transport of compounds from plant organs towards plant drains, roots and stalks (Epstein and Bloom, 2006). This may have resulted in competition between drains and sources, reflecting in a small biomass production. Roots are responsible by the gathering of crucial reserve substances for the development of *J. curcas*. Therefore, when the plants were cultivated under unfavorable environment for their development, modifying physical and chemical proprieties of the soil, detrimental effects such as reduction in biomass production were expected, impairing plant growth as well as distribution of compounds among plant organs.

In the present work, native microbiota had probably higher importance concerning *J. curcas*. growth on the topic of dry biomass production. Bacteria with the ability to solubilize phosphate through the production of organic acids or phosphatases, may indirectly make this nutrient available for plant absorption, once these bacterial secreted compounds may transform phosphorus solubility (Marschner et al., 2012). Bacteria from the native microbiota may have being associated with the plant adaptation observed in the present work, and their isolation to constitute part of a future new inoculum has to be considered. On the contrary, given the results concerning Phosphate Solubilizing Bacteira from the inoculum, in the case of biomass production, there was no evidence of these microorganisms as being auspicious growth promoting microorganisms for *J. curcas*.

Analysis of phosphorus content in *Jatropha curcas* leaves

The vast majority of plants showed an increment in phosphorus contents until the end of the experiment, suggesting that bio-solubilization of nutrients by the inoculum or production of phosphatases by native microbiota occurred. Dieng et al. (2014 and 2015), observed that microbial biomass and phosphorus and nitrogen contents are higher in soils planted with *J. curcas*. However, in treatments SWRWI, S135RWI and S27RWI the content of phosphorus in plant leaves decreased. The values observed for the gathered P in SWRWI plants at 240 days were lower than the higher values of 3.2 g/Kg observed previously by Lima et al. (2011). However, the authors also observed reduction in the values of gathered phosphorus at the end of the experiment. Similar to what was proposed by Laviola (2009), nutrients contained in leaves, which are transportable by the phloem, may have been re-mobilized towards growth sites before leaves dropped. This may also have helped plants in SWRWI to persist in a nutrient deficient soil. Besides that, the production of phosphatases and other soil enzymes by the plant and by the native microorganisms was most likely satisfactory, in the pursuit of P and other minerals from other soil sources. Considering in the present work the soil used was meager in nutrients, it is feasible that plant characteristics, such as good absorption ability, mobilization and re-utilization of nutrients, specifically with positive adaptive relations with the native microbiota, had been the differentials for the better results obtained in SWRWI. According to Resende et al. (2011),

soil native microbiota may provide nutrients that support the initial plant growth. This probably happened with *J. curcas L*, once there was an increment in the accretion of phosphorus in leaves until 120 days. The positive association between nutrient accretion and biomass production was previously observed by Freiberger (2010), with some plants of *J. curcas* that showed a satisfactory growth size. According to Malavolta (1989), plants are unable to utilize more than 10% of the total P, due to the phenomenon of phosphorus fixation. In order to make fixed phosphorus soluble and assimilable by the plant, the involvement ofsome soil bacteria and fungi species that are able to promote plant growth is required. Therefore, the availability of phosphorus due to the activity of native microorganisms may have also influenced the development of plants in the absence of inoculum and rock dust.

For other treatments analyzed, the gathering of phosphorus was higher than in SWRWI plants only starting at 120 days, suggesting that besides the pre-existent quantities of such nutrient, a bio-solubilization of phosphorus from the rock dust (and its absorption by the plants), promoted by the native microbiota and/or by the inoculum may have occurred, starting during that period. However, despite showing higher values for phosphorus foliar contents than SWRWI, plants did not show the same efficiency for nutrient utilization, evidencing the possibility of physiological problems in nutrient re-distribution at the beginning and during the development period. This was also observed by Pacheco et al. (2012) and Morais (2010). Lima et al. (2011) quantified the re-distribution of macro- and micro-nutrients in leaves of *J. curcas* as a function of the phenological stage and concluded that nutrients P, K, Cu and Zn are intensely re-distributed from older leaves towards the most younger tissues in *J. curcas*, while there is a low re-distribution of N, Ca, Mg, Fe and Mn. Probably, the negative effects from rock dust, in relation to the deficient nutrient releasing, influenced during the first periods analyzed, reflecting in the processes of mobilization and allocation of phosphorus and probably other nutrients within the plant. With a little demand for plant growth, reflected in a smaller gathering of biomass, phosphorus and probably other nutrients gathers in plant leaves creating a concentration effect. The values observed, greater than native bacteria treatment and considered high for phosphorus according Lima et al. (2011), involves plant and microbiota adaptive matters to the inoculated and rock dust environment during this period. However, the physiological and morphological consequences for the plant facing soil physical modifications and initial competition for phosphorus or other nutrients, specially competing with the inoculated microorganisms, may have reflected in the small growth as in plants from SWRWI until 120 days. In plants where the phosphorus concentration in leaves was slight, without bacterial competition, a higher growth was observed, thus indicating a better utilization of nutrients. Regarding the use of rock dust as the only soil fertilization material for *J. curcas*, most of the results were unfavorable and therefore this parameter has to be re-evaluated under a perspective of being used together with another nutrient source.

Microbial enzymatic activity

Soil enzymatic activity values were low. Considering the data interpretation established by Lopes et al. (2013), for acid phosphatase, the critical levels observed for soil with or without rock dust were <40%, thus considered low. According to these authors the levels considered adequate for plant development are 80%, 1160 µg of p-nitrophenol.g^{-}

^{1}soil.h^{-1}, for this enzyme. However, soils differ with regards to their physical, chemical and biological properties, having variable levels of enzymatic activity. The absence of significance among the main effects and interactions between rock dust dosages and the evaluation periods for treatments, with and without inoculation, revealed that enzymatic behavior of phosphatase was different in both cases (with and without inoculation). Phosphatase exhibited the highest values, indicating the relevance of this enzyme for nutrient cycling in the studied soil, as previously observed by Silva et al. (2009). Soils have a phosphorus reserve that allows the initial development of crops (Resende et al., 2011), but the availability of this nutrient is reduced. Chemical analysis of the samples showed a low phosphorus concentration in the soil, which may have influenced the higher values of phosphatase. These values, statistically different from the other treatments, were found in SWRWI, as expected, since this treatment did not receive any nutrient source, thus compelling microorganisms and/or plants to search for nutrients from different sources in the soil, by producing enzymes. The restricted availability of phosphorus in the soil resulted in a requirement to increase the production of phosphatases, thus indicating a negative correlation for this factor. In general, the values for phosphatase activity were low, but the quantity of phosphorus in the soil was high, when observing the foliar concentration of P in *J. curcas* leaves, thus suggesting bio-solubilization of rock dust by microorganisms. At the end of the experimental period, some inoculated plants matched or surpassed the height of SWRWI plants, as in S81RI, showing the phosphate availability from rock dust, as previously mentioned. However, undesired effects during the beginning of plant growth, caused by the addition of rock dust, with a slow nutrient release (Knapik and Angelo, 2007), and the consequences of a possible competition from phosphate solubilizing bacteria with the native microbiota (Richardson and Simpson, 2011), may have been responsible for the reduced plant growth. Apparently, these plants experienced the effect of the nutrient concentration in their leaves, not being able to mobilize these nutrients and distribute them in an adequate manner to different organs.

The low values of enzymatic activity observed in this study suggest that phosphorus contents and possibly other nutrients contents, associated with other soil modifications created by the addition of rock dust, interfered with microbial activity. Soil organic matter quantities may have also influenced the low enzymatic values observed, but when observing the phosphorus contents in leaves of *J. curcas* it is apparent that microorganisms and plants had enough availability of this nutrient and probably others, obtained from rock dust, with no need for a higher production of extra-cellular enzymes. Consequently, microbial activity was a preeminent factor for plant growth in a soil where rock dust was added or when phosphate solubilizing bacteria were used at the beginning of the growth period, although with a delayed effect.

Concerning *J. curcas* plants in SWRWI, the native microbiota was probably responsible for the accessibility of nutrients to plants, with a reflex in higher value for biomass production when compared with non-inoculated treatments. The best values for phosphatase activity and other enzymes were observed in SWRWI plants. Even though the values of enzymatic activity were higher, these values were not considered high. Hence, it is presumed that the native microbiota used other alternatives to obtain nutrients, probably by the production of organic acids, suggesting that native microbiota assumed important functions for soil sustainability and showed potential to promote growth of *J. curcas* plants.

Future research should focus on more specificities of resident microbiota activity in plant growth promotion and the isolation of these microorganisms to produce other inocula, to be tested in various plants.

CONCLUSIONS

Phosphatase activity of native soil microorganisms was higher than observed in bacteria inoculum. Phosphorus and possibly other nutrients contents and the addition of rock dust promoted microbial activity. The highest content of phosphorus, 2.49, and dry biomass occurred in the presence of soil-resident microbiota only up to 120 days, 70.45 in leaves; 73,98, in roots, and 105.44, in stalks. The soil samples under the influence of only resident microbiota showed the higher enzymatic activity results. The highest values were observed for the activity of the acid phosphatase. Phosphatases had values of 130.69 µg at 30 days, 155 µg, at 120 days, and 122.62 µg of p-nitrophenol.g-1soil.h- 1, at 210 days. Soil organic matter quantities may also have influenced the low enzymatic values observed. Phosphatase exhibited the highest values, indicating the relevance for nutrient cycling. Greater dry biomass was produced when the rock dust was not used, without Phosphate Solubilizing Bacteria, suggesting the relevance of the native microbiota in plant growth promotion. The isolation of native microorganisms and microbial activity assays will reveal other specific potential for bacteria and fungi promoting plant growth, as for example organic acid production; such microorganisms will ultimately be employed to constitute new and improved inocula.

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CONFLICTS OF INTEREST

The authors declare no conflict of interest.

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