CARACTERIZAÇÃO CULTURAL E FENOTÍPICA, E AVALIAÇÃO DE TOLERÂNCIA A ESTRESSES AMBIENTAIS DE RIZÓBIOS NATIVOS COLETADOS DE NÓDULOS RADICULARES E SOLO RIZOSFÉRICO DE LEGUMINOSAS ARBÓREAS NA CAATINGA

PHENOTYPICAL AND CULTURAL CHARACTERIZATION, AND EVALUATION OF ENVIRONMENTAL STRESSES TOLERANCE OF NATIVE RIZHOBIA COLLECTED FROM RADICULAR NODULES AND RIZOSPHERIC SOIL OF LEGUMINOUS TREES IN CAATINGA

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Resumo: Esta pesquisa tem como objetivo apresentar os resultados dos testes de isolamento, identificação, caracterização fenotípica e tolerância ao estresse ambiental de rizóbios coletados de nódulos radiculares e solo rizosférico de leguminosas arbóreas em dois períodos distintos, estações seca e chuvosa, em área semiárida no Nordeste do Brasil (Crateús-Ceará). Este estudo argumenta que a análise de características fenotípicas são ferramentas valiosas, como etapa inicial para posterior análise de sua genética, na identificação de características adaptativas, ajudando pesquisadores na identificação acurada de cepas ideais, dentre uma ampla diversidade, de variedades promissoras de rizóbios capazes de responder às mudanças climáticas, estando possivelmente melhor equipados para aplicação agrícola em um mundo mais seco. Esta pesquisa confirmou a presença de Rhizobia na área de estudo, em que 92,42% dos isolados apresentaram crescimento rápido, e 52,24% de acidificação do meio de cultura 79, enquanto resistência a altas temperaturas (45C), e altas concentrações de sal (5%) foram observadas em 84,93% e 90,75% dos isolados, respectivamente. Foi observado a formação de grupos funcionais de acordo com a estação chuvosa, e as plantas-iscas utilizadas, o que pode indicar promissores indícios de coevolução entre bactérias e leguminosas, e o ambiente no bioma Caatinga. Futuros estudos são necessários para validar a viabilidade econômica e aplicação produtiva dos resultados.

Palavras-chave: Soluções Baseadas na Natureza; Mudanças Climáticas; Estresse Ambiental; Biodiversidade; Caatinga;

Abstract: The objective of this research was to present the results of the isolation, identification, phenotypic characterization and environmental stress tolerance of rhizobia collected from root nodules and rhizospheric soil of tree legumes in two distinct periods, dry and rainy seasons, in a semi-arid area in the Northeast of Brazil

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(Crateús-Ceará). This study argues that the analysis of phenotypic characteristics is a valuable tool, as an initial step for later analysis of their genetics. The identification of adaptive characteristics may help researchers in the initial identification of ideal strains from a wide variety of promising varieties of rhizobia capable of responding to climate change, possibly being better equipped for agricultural application in a drier world. This study confirmed the presence of Rhizobia in the study area, in which 92.42% of the isolates showed rapid growth, and 52.24% acidification of the culture medium 79, while resistance to high temperatures (45C), and high concentrations of salt (5%) were observed in 84.93% and 90.75% of the isolates, respectively. It was observed the formation of functional groups according to the rainy season, and the bait plants used, which may indicate promising signs of coevolution between bacteria and legumes, and the environment in the Caatinga biome. Future studies are needed to validate the economical feasibility use, and rich application, of results.

Keywords: Nature-Based Solutions, Climate Change; Environmental Stress; Biodiversity; Caatinga;

INTRODUCTION

Bacterial species in the genera Rhizobium are capable of forming a symbiotic association with legume providing reduced nitrogen in exchange for carbon and energy from the host plant; furthermore, Rhizobia have a wide phenotypic and taxonomic diversity and are distributed among several groups worldwide (FERNANDES JÚNIOR et al. 2012). The literature reports the importance of rhizobium strains evaluation for different pH ranges, salt concentrations and high temperatures tolerance, as well as intrinsic resistance to antibiotics and utilization of carbon sources as essential criteria for selection of promising future inoculants (HERNÁNDEZ et al. 2012). Commonly, such assessments may include the demonstration of the ability of rhizobia to form nodules with a wide range of legumes and the characterization of the Biological Nitrogen Fixation activity –BNF – one of the most critical interactions that may occur in the soil once rhizobia strains play a fundamental role in the sustainability of agroecosystems (FERREIRA COSTA, 2010). However, the rhizobia-legume association efficiency is not only conditioned by the combination of bacteria strains and the cultivated variety (FERREIRA et al. 2012). By definition, the host spectrum is quite variable among species of rhizobia and legumes, although, overall specificity has been reported between species (SMITH and GOODMAN, 1999). Besides, its efficiency has to do with environmental conditions where the symbiosis may occur (FERREIRA et al., 2012). Studies prove that the ability of legumes to form nodules is directly linked with the magnitude, distribution and diversity of rhizobia counterparts in a given soil and, also, related to the existence of environmental factors that may negatively act on the BNF process, such as salinity and high temperature stresses (BALA et al., 2003). These significant environmental stress factors may be manifested by the inhibition of the nodulation processes as well by the decreasing colonization of legume roots (VENTORINO et al., 2012). In turn, soil pH may also act as a limiting rhizobia-legume symbiosis factor because it affects the propagation and survival of rhizobia as well the nodulation process - including the nitrogen fixation(RUIZ-DÍEZ et al., 2012). Soils in semiarid regions are generally saline, and present limitations regarding acidity and fertility, these factors coupled with high temperatures, toxic levels of aluminum and presence of antibiotics produced by other microorganisms may negatively affect rhizobia (SOUSA et al.,2013; FERNANDES et al., 2013).

Based on that, this study focus on the isolation, identification, phenotypic nd cultural characterization, and environmental stress tolerance tests of rhizobia strains collected from root nodules and rhizosphere soil in two periods, dry and rainy seasons, in a semiarid area in the Northeast of Brazil. Although legume biodiversity is concentrated in tropical regions, the majority of studies on legume nodulating bacteria are focused on cultivated leguminous plants from temperate regions, and most of the information on this topic derives from studies conducted in those regions. Studies in semiarid regions in the tropical world may be useful for the identification of symbionts with diverse characteristics enabling new knowledge, uses and the identification of strains adapted to extreme conditions and in turn viable to meet the challenges posed by climate change.

MATERIALS AND METHODS

The field site for the soil samples and root nodules collection was the Private Natural Heritage Reserve (RPPN - acronym in Portuguese) in the "Serra das Almas" Natural Reserve (05°00 'to 05°20'S and 40°48-41°12'W), belonging to the conservation NGO "Associação Caatinga", located in the Northeast region of Brazil, in the city of Crateús in the State of Ceará, Brazil. The reserve is situated 390 km from Fortaleza, the capital State of Ceará. The climate is semiarid with an average rainfall of 881mm/year, distributed from January to April (ARAÚJO and SILVA, 2007). The root nodules and the rhizosphere soil samples were collected in a preserved area of native vegetation (Caatinga) arbitrarily selected in the Natural Reserve after the recognition of areas with fully established native legume trees; we transferred the collected samples to a plastic container that was then sealed, identified, transported and stored in a greenhouse at room temperature (average 28°C), we kept it protected from the sun in a dry place. Given the fact that the methodology used for isolation, identification and phenotypic characterization of rhizobia is notoriously known, wide and extensively used, and in order to keep this section of the study objective, as the whole process is accuratelly described in the results; we only cited the sources consulted. We conducted controlled experiments in a greenhouse to capture rhizobia in accordance with Rumjanek (1999). The isolation was performed according to Hungria et al., (1997), and later, we evaluated cultural characteristics in culture medium 79 with Congo Red and Bromothymol Blue indicators according to Vincent (1970). After that, we performed cluster analyses according to the Jaccard coefficient, and finally, we evaluated the isolates for salinity tolerance in culture medium 79 at NaCl concentrations of 1%, 3%, and 5%, as Fred and Waksman (1928), and then, we checked isolates resistance to high temperature at increasing temperatures of 41°C, 43°C, and 45°, for a period of 48h. We designed all treatments utterly randomized with three replications each.

We collected the rhizosphere soil from the following species of native legume trees: (i) *Angicos-Anadenanthera macrocarpa (Benth.) Brenan*; (ii) *Mororó-Bauhinia cheilantha*; (iii) *Catingueira-Caesalpinia pyramidalis Tul*; (iv) *Mulungu-Erythrina fusca*; (v) *Sabiá-Mimosacae salpinifolia*; (vii) *Jurema-branca-Mimosa acustistipula*; (viii) *Jurema-preta-Mimosa tenuiflora*; (ix) *Emburana-Amburana cearensis Fr. All.*; in two distinct periods, first in the rainy season of 2008, and later in the dry season of the same year. We captured rhizobia, present in root nodules and rhizosphere soil, from the following bait plants: (i) *Siratro-Macroptilium atropurpureum*; (ii) *Cowpea-Vigna unguiculata L. Walp*.; (iii) *Pigeonpea-Cajanus cajan*; and (iv) *Mimosa-Mimosa pudica L.*; which are species described in the literature as promiscuous, and for this reason, ideal for initial studies capturing rhizobia in a controlled-environment.

RESULTS AND DISCUSSION

The study presents the results of the isolation, identification, phenotypic and cultural characterization, and environmental stress tolerance analysis of rhizobia collected from root nodules and rhizospheric soil of tree legumes in two distinct periods, dry and rainy seasons, in a semi-arid area in the Northeast of Brazil. (Crateús-Ceará). This study argues that the analysis of phenotypic characteristics is a valuable tool, as an initial step for later analysis of their genetics, in the identification of adaptive characteristics (FERREIRA COSTA et al., 2010). It may help researchers in the accurate identification of ideal strains from a wide variety of promising varieties of rhizobia capable of responding to climate change, possibly being better equipped for agricultural application in a drier world. This study confirmed the presence of Rhizobia in the study area.

The present study showed a very little presence of root nodules in leguminous trees under natural conditions, as opposed to a study conducted in Pakistan, by Shahzad et al., (2012), which pointed broad nodulation in natural conditions. Granted that, it is essential to mention that we only observed field nodulation in just one of the species (*Mimosa caesalpinifolia*), and during the rainy season, as shown in the Figure 1. In figure 2, we present images of the area in the rainy and dry season for a better understanding of the climatic changes suffered between seasons.

Figure 1. Root nodule, in natural conditions, in the shallow root system of "Sabiá"(Mimosa caesalpinifolia Benth.); Site: Private Natural Heritage Reserve (Reserva Particular do Patrimônio Natural - RPPN) "Serra das Almas" Natural Reserve. Crateús, Ceará, Brazil (2008);

Figure 2. Field site collection - rainy season on the left and dry season on the right; "Serra das Almas" Natural Reserve, Crateús, Ceará, Brazil (2008);

Nonetheless, as a result of greenhouse and laboratory experiments, Table 1 describes in detail the obtained results regarding the presence and formation of rhizobium nodules under natural and laboratory conditions their distribution, by native plant and bait plant, as well as season (dry and rainy). We obtained 776 isolates that have been presumptively identified as rhizobia after being captured by four bait plants: *Macroptilium atropurpureum* (152 isolates; 62 from the rainy season; and, 90 from the dry season); *Vigna unguiculata L. Walp* (278 isolates; 156 from the rainy season; and, 122 from the dry season); *Cajanus cajan* (190 isolates; 126 from the rainy season; and, 64 from the dry season); and, *Mimosa pudica L.* (146 isolates; 80 from the rainy; and, 90 from the dry season; respectively). The cultural and phenotypic characterization of these isolates where used as an initial step to find significant differences used for grouping the strains by the Jaccard method.

We chose these specific bait plants due to their intrinsic characteristics; *Vigna unguiculata L. Walp*, so as *Cajanus cajan*, and *Macroptilium atropurpureum* are described as promiscuous in the literature; i.e., they have the ability to form nodules with an extensive range of bacteria (FERNANDES JÚNIOR, 2011; 2012), while the genera *Mimosa* has strategic value being described as broad and complex, where many species can be observed in different types of savannas, such as "Caatinga" and "Cerrado". Thus, the use of these species was based on their ability to provide a broad representation of the biodiversity of rhizobia in semiarid soils, as its strains seem to have comprehensive degradative and metabolic capabilities that allow them to grow on various soil and rhizosphere substances, many of which are still unknown (FERREIRA COSTA, 2010; ORMEÑO-ORRILLO&MARTÍNEZ-ROMERO, 2013), specially in semiarid soils in the Caatinga.

Table 1. Presence and formation of rhizobium nodules under natural and laboratory conditions the distribution, by native plant and bait plant, as well as season.

Leguminous Trees in the Natural Environment	Soil Samples	Dry Season (Bait Plants)				Rainy Season (Bait Plants)			
		Vignaunguicu lata L. Walp	*Cajanus cajan*	*Macroptilium atropurpureum*	*Mimosa pudica* L.	*Vignaunguicu lata* L. Walp	*Cajanus cajan*	*Macroptilium atropurpureum*	*Mimosa pudica* L.
Rhizobia Nodule - *Mimosa caesalpinifolia* (Sabiá)		x	0	0	0	x	0	0	0
Caesalpinia pyramidalis Tul. (Catingueira)	1	x	0	x	0	x	x	x	x
Bauhinia cheilantha (Mororó)	2	x	x	x	x	x	x	x	x
Mimosa acustistipula (Jurema-branca)	3	x	x	x	x	x	x	x	x
Erythrina fusca (Mulungu)	4	x	x	x	x	x	x	x	x
Mimosa tenuiflora (Jurema-preta)	5	x	x	x	x	x	x	x	x
Anadenanthera macrocarpa (Bent.) Brenan (Angico)	6	x	x	x	x	x	x	x	0
Mimosa acustistipula (Jurema-branca)	7	-	-	-	-	x	x	x	x
*Amburana cearensis*Fr. All. (Emburana)	8	x	x	x	x	x	x	x	x
Mimosa caesalpinifolia (Sabiá)	9	x	x	x	x	x	x	x	0
Erythrinafusca (Mulungu)	10	-	-	-	-	x	x	x	x
Amburana cearensis Fr. All. (Emburana)	11	x	x	x	x	x	x	x	0

Legend: (x) Nodules presence; (0) No nodules formation; (-) Early stage bait plant death;

We are in compliance with Marra (2012) regarding the use of simple initial studies of cultural characterization and stress tolerance of rhizobia in natural and controlled environments. By itself, it is a powerful tool that provides crucial initial data and information for the identification and grouping of microorganisms, saving money and time for further and tailored studies. Instead, the DNA analyzes are more expensive, take longer and usually require trained personnel, which often prevents the study of rhizobia in semiarid regions of developing countries. The limiting reality faced during this study.

Naturally, the soils and the environmental conditions, in which these strains developed are also essential elements since they may act as limiting factors, not only affecting biodiversity, but also the nitrogen fixation, in the presence of other bacteria (FERREIRA COSTA & MARTINS, 2009). For this reason, we support the value of studies based on phenotypic criteria and tolerance response to environmental stresses as useful steps to identify a wide diversity of rhizobia in semiarid soils that otherwise would remain unknown. This point of view is also shared by Chagas Júnior et al., (2009), although, according to Rocha et al. (2012) phenotypic characterization may present limitations to indicate diversity. Limitations that we recognize and account for, as phenotypic features cannot be isolated in similar groups as they may have many different responses, not entirely triggered by environmental and stress conditions, or accounted for in a laboratory. However, this nature of study reminds us that isolates response to environmental stresses may be a helpful tool to support the identification of specific bacterial functional groups.

In this sense, the analysis of semiarid soils may contribute to improving the knowledge regarding soil-rhizobia interactions in fairly studied situations, because it goes beyond bacterial-plant interactions.

Many interesting results came from the soil analysis. The soil sample analysis demonstrated the presence of medium to high concentrations of Mg (cmol c/kg), and Ca (cmol c/kg), which were considered a positive indicator for the BNF process in this environment; notwithstanding, the presence of significantly high levels of salinity, naturally occurring in this soil, may negatively affect the balance of absorption of other elements by the plants, affecting the nitrogen fixation by the bacteria, potentially hindering the emergence and growth of plant roots, and ultimately reducing the points of infection by rhizobia, as defended by Bouhmopuch et al. (2004). Although we cannot affirm this as the primary cause of the limited presence and formation of Rhizobium nodules under natural conditions, we believe these conditions offered a glimpse of knowledge to better understanding the reasons behind the reduced presence of nodules in the field. Besides, we observed low concentrations of K (cmol c/kg), and P (mg/kg) in the soils, characterizing it as poor soil. On the other hand, we also observed low levels of Al concentrations (cmol c/kg), which represents a less adverse effect on some steps of BNF process. Notwithstanding, the concentrations of elements present in this soil, are described in the Table 2, and, although its analysis was not sufficient to explain how the concentrations of these elements may affect the interactions bacteria-plants interactions, and their response in the fixation of nitrogen, it helped us to better understand the environment, and the diversity of ways of living, possibly indicating coadaptation to respond to local environmental conditions and stresses.

Table 2. Soil analysis; Private Natural Heritage Reserve (PRNP) in "Serra dasAlmas" Natural Reserve, Crateús, Ceará, Brazil (2008).

Soil Samples	P	K	Ca	Mg	Na	C	Al	E CaCO3	pH H_2O	pH KCl
	mg/kg	Cmolc/kg	Cmolc/kg	Cmolc/kg	Cmolc/kg	g/kg	Cmolc/kg	g/kg		
1	0,51	0,26	1,00	0,90	0,46	7,50	0,35	22,00	4,93	4,20
2	1,67	0,34	4,75	0,70	0,31	15,90	0,10	33,00	6,33	5,53
3	0,74	0,30	1,40	1,45	0,31	12,42	0,10	32,00	5,50	4,53
4	0,88	0,36	2,50	0,85	0,29	10,14	0,15	29,50	5,33	4,62
5	0,88	0,31	1,95	3,15	0,30	15,42	0,20	28,00	5,51	4,61
6	1,77	0,32	3,75	2,05	0,36	14,88	0,10	33,75	5,20	4,82
8	4,45	0,30	5,50	2,45	0,30	16,20	0,10	46,50	6,74	6,03
9	4,19	0,29	5,95	2,10	0,35	16,62	0,05	30,50	6,60	5,96
11	2,01	0,40	5,10	2,25	0,35	16,62	0,05	30,50	6,19	5,49

Soil samples 7, 10, and rhizosphere soil sample with field nodules were not possible to be analyzed due to their small quantity available, not allowingt replication tests;

Medeiros et al., (2009) studying morphological diversity of rhizobia from cowpea in another semiarid area of northeastern Brazil found the dominance of fast-growing strains. This study may corroborate their results as it reports an extensive collection of rhizobia characterized by fast and high-speed growth, under similar conditions (Figure 3; and, Figure 4). We may understand this feature as a survival strategy since both areas share similar characteristics. Besides, Santos et al., (2007) present quite similar results in another study, which relates fast growth rate as resistance to drought. The unique point in our study, in the higher presence of high-speed growth strains, compared to similar studies. It may indicate a particular response to specific environmental conditions and stresses present in the area, with possible promising varieties of rhizobia capable of responding to climate change, possibly being better equipped for agricultural application in a drier world, and economical use feasibility, as well as agricultural application of the results.

Figure 3. Growth rate of rhizobia isolates - in days.

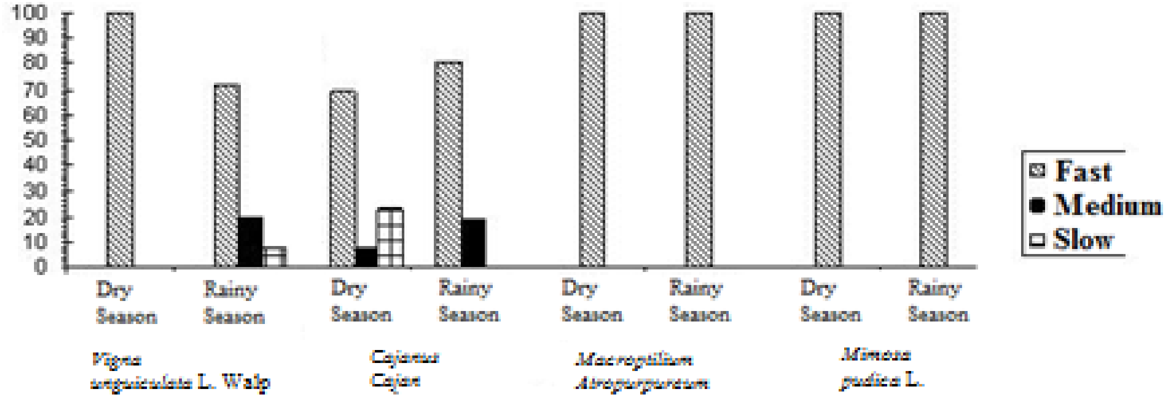
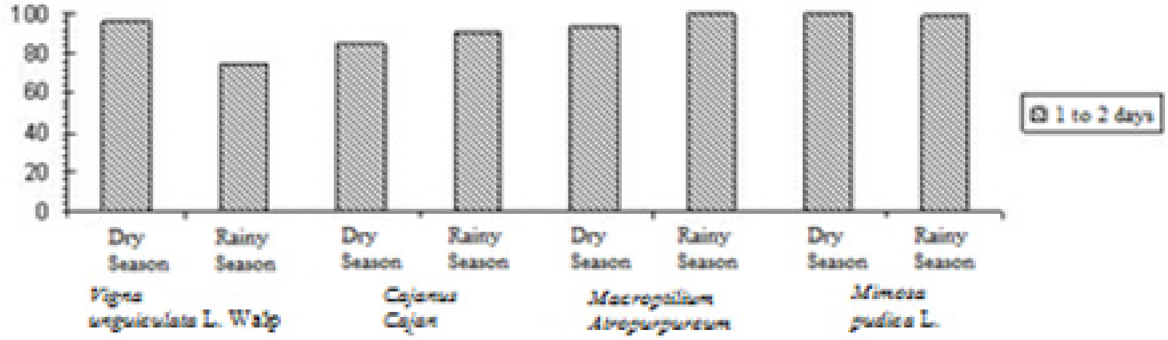
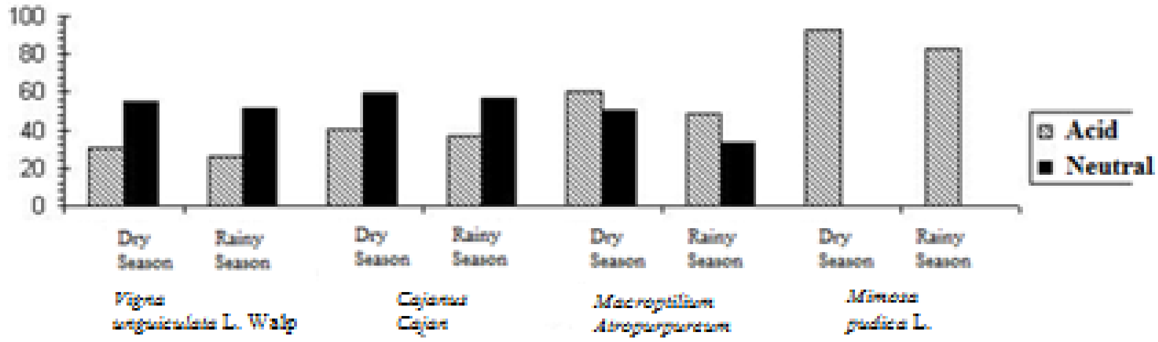


Figure 4. Growth pace of rhizobia isolates.



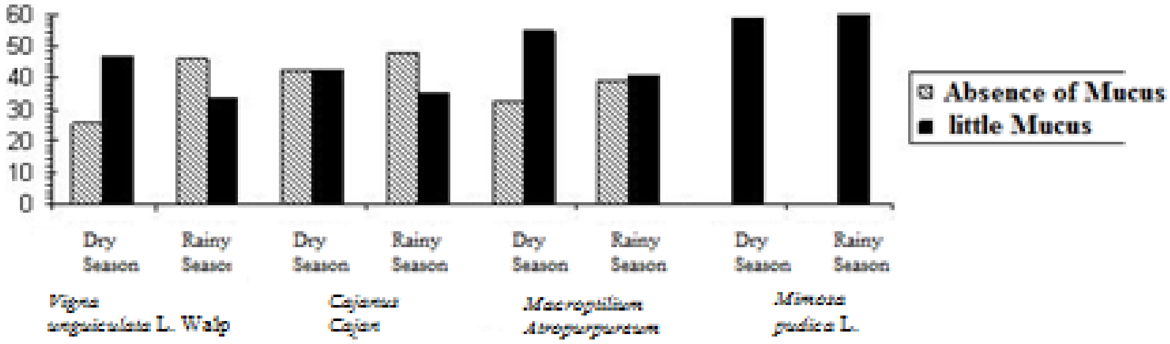
Another exciting feature that emerged from this study was the ability of the analyzed strains to alkalize the culture medium. According to Xavier et al., (2007), it may represent a selective advantage over natural conditions in tropical areas. A characteristic that is usually present among strains found in areas with high temperature, and soils with low levels of pH, that facilitates their survival under harsh conditions. Regarding his assumption, according to our results, we are not completely in accordance with them, as we believe that the production of mucus could not only represent a strategy used by rhizobia to alter the soil pH -as most of our isolates showed an acidic and neutral medium reaction in vitro (Figure 5). It might be inherent characteristics of the strains found in the area in response to their interaction with other microorganisms as well as response to the bacterial-plant interactions. These results demand further studies to offer a complete view of the bacterial-plant response to environmental conditions, and microbial competition in semiarid soils.

Figure 5. pH medium response produced by isolates of rhizobia.



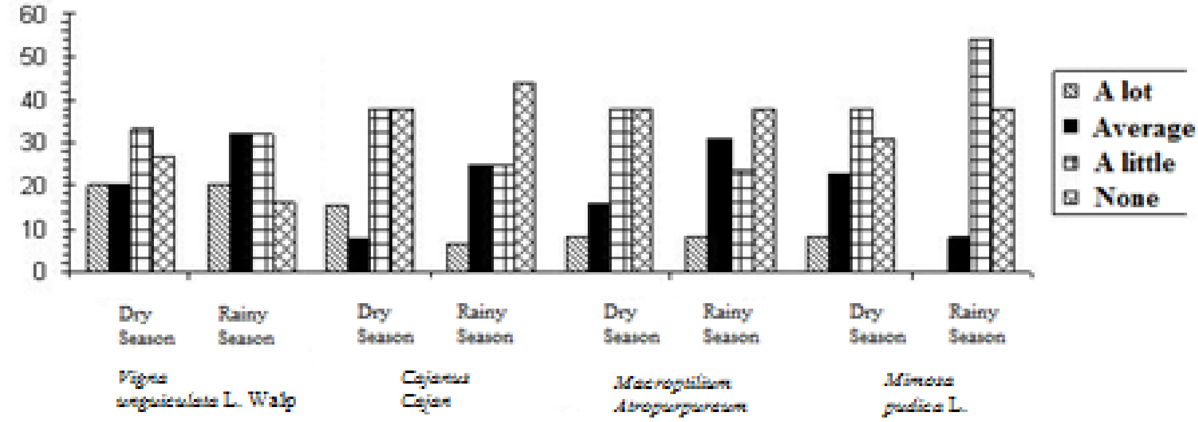
According to Fernandes Júnior et al. (2012) the higher the production of mucus the more significant the tolerance to high temperatures and salinity. As a result, we presented another up-and-coming feature found among the obtained strains. We observed an evident lack of correlation between temperature tolerance and mucus production in the strains studied. We observed the dominance of isolates with little or no production of mucus with relatively high tolerance to environmental stresses (Figure 6).

Figure 6. Mucus production by isolates of rhizobia.



We assume the possibility of existence of isolates with dry mucus, and therefore no elasticity, to present a higher resistance to high temperatures; a factor that ensures their survival in arid conditions; now, in complete accordance with Xavier et al., (2007). This hypothesis may be valid according to our results; however, we also could assume that high temperatures may not be a limiting factor after all, as these strains are adapted to local environmental conditions (biotic and abiotic). Once natural conditions will be "optimal" -as we Humans understand it -, the strains will modify, or "requalify", their behavior and response. Notwithstanding, more studies are needed regarding its activity, and stress response in this environment. It is possible that low mucus-producing strains presented a variation of tolerance to environmental stresses, but we are not enterile convinced of that. Among the strains studied we observed a considerable variation in the characteristics of their mucus related to its elasticity; despite the high prevalence of isolates with dry mucus (little or no elasticity) (Figure 7).

Figure 7. Elasticity of the mucus producedbyisolates of rhizobia.

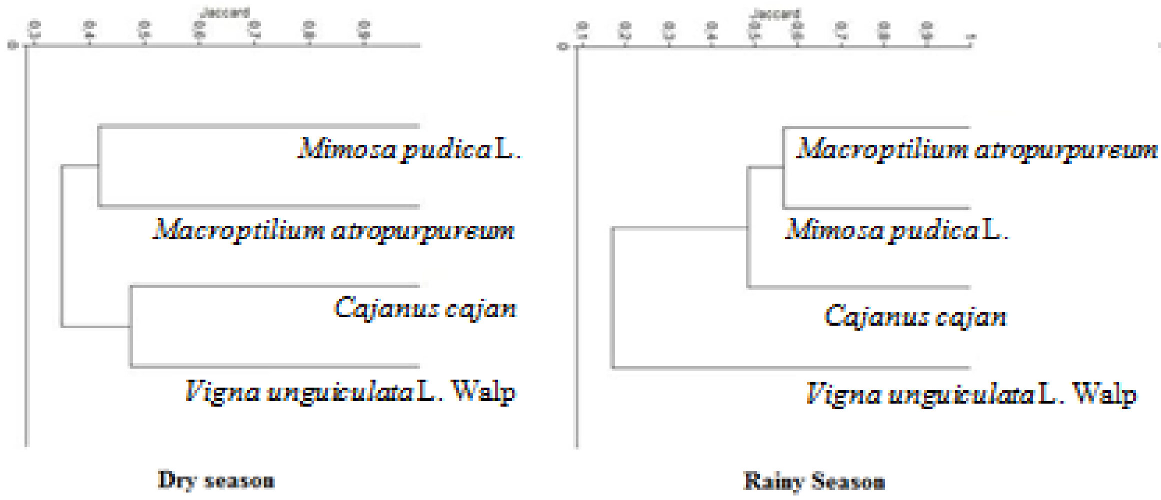


The Cluster Analysis

The use of different clustering methods in the evaluation of biodiversity is of crucial importance to evaluate the future genetic divergences between strains of rhizobia. In this study, we used the Jaccard coefficient, following extensive and representative previous literature. The knowledge about the diversity among a group of parent bacteria of any species is of great importance, especially in the identification of those combinations having a greater heterotic effect. When such combinations are taken as a basis, the probability of recovering superior genotypes in segregating generations is greater (ROTILI et al., 2012).

Granted that, upon completion of the cultural and phenotypic characterization we, then, performed a cluster analysis to generate dendrograms of similarity based on host cultural groups of rhizobia associated with the bait plants and according to seasons by the use of the Jaccard coefficient. For the rainy season, we observed the formation of four colony groups with 45% similarity; the distance between isolates ranged from 15% to 100%; in the rainy season, *Vigna unguiculata* L.Wal presented the group of isolates with the lowest similarity rate, when comparing isolates from the other bait plants. For the dryer season, again; we observed the formation of four well-defined groups. However, this time, the observed similarity reached 50%, while the distance among groups, ranged from 35% to 100%; for the dryer season, wondrously, Macroptilium atropurpureum, and Mimosa pudica L. presented a higher rate of similarity between the groups, as opposed to *Vigna unguiculata* L.Walp, and Cajanus cajan that showed higher rate of similarity between each other. It may confirm studies about the overall specificity that has been reported between species in the literature, as described by Smith & Goodmand (1999). Also, we found that the season influenced the bacteria grouping. One Hundred and Twenty-One cultural groups (121) were formed with levels of similarity between 50-60% (Figure 8). The use of this clustering method, supported an of indication of microbial biodiversity, being essential to support future genetic studies between strains of rhizobia. As a result, we selected representative isolates of each group for evaluation against environmental stresses such as increasing temperatures, and salinity levels.

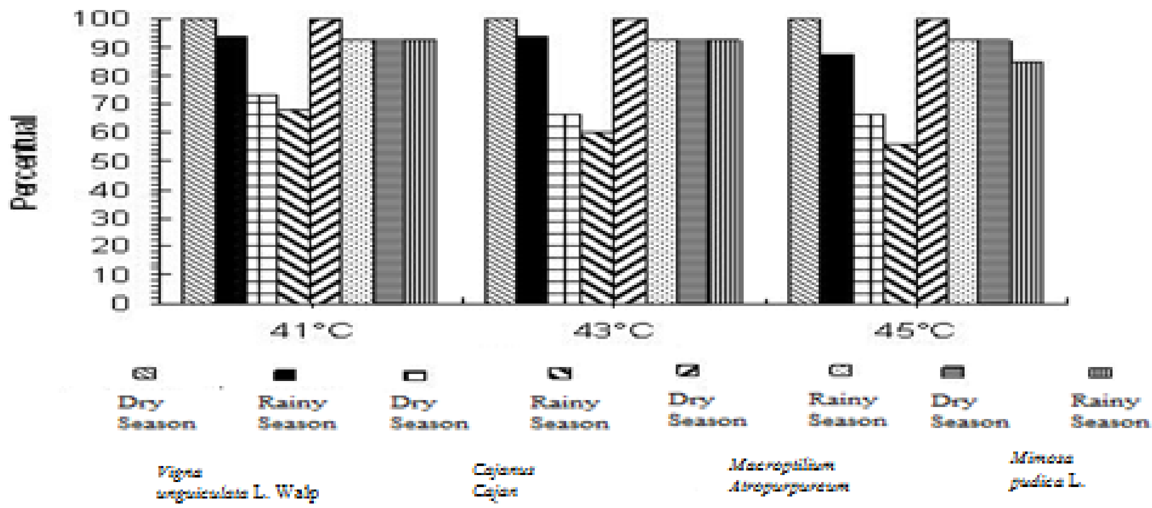
Figure 8. Dendrograms of similarity based on bait plants associated with cultural groups of rhizobia captured from soil samples collected in the rainy and the dry season in the Private Natural Heritage Reserve in the "Serra das Almas", Crateús/Ceará, Brazil.



The results from the studies performed demonstrated a higher tolerance of the selected strains to increasing temperature rates, in vitro (Figure 9). The groups of isolates obtained from *Cajanus cajan*, and *Vigna unguiculata L. Walp* showed the highest rates of tolerance to increasing temperatures (with 100% of survival) during rainy and dryer seasons equally. This result may reflect the potential for agricultural use, and rich application of the results, such as the use of these isolates in semiarid areas to recovery degraded soils and the development of a complex network of interactions which stimulates the binding of carbon in semiarid soils, helping to mitigate the impacts of future climate change.

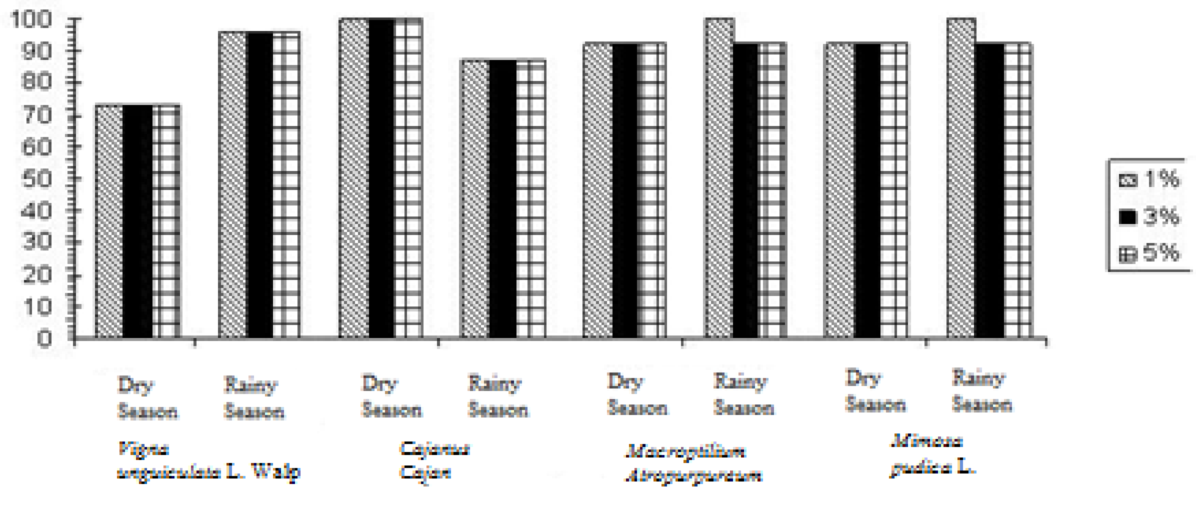
The isolates showed ability to grow over a broader range of pH, higher levels of salinity and temperature. Nevertheless, the presence of antibiotics, and diverse carbon sources utilization may be responsible for their saprophytic persistence in the soil, as corroborated by the studies of Fentahun et al., (2013). Notwithstanding, we call attention to the risks of the degradation of semiarid ecosystems in the Caatinga biome, which may negatively influence, reducing the density and diversity of these bacteria in semiarid soils. A point of view also shared by Moreira et al. , (2010).

Figure 9. Resistance to high temperatures (41, 43 and 45° C), according to the season.



Uyanöz & Karaca (2011) studying the effects of different salt concentrations and rhizobium inoculation on the growth of dry bean, found that population of R. tropici strain CIAT899, and natural Rhizobium decreased under higher salinity levels. Nonetheless, the population count of the CIAT899 strain was inversely proportional to the salt concentration. In the present study, the observed effect of seasons in the diversity of rhizobia showed different behaviors and responses among strains when evaluating salinity and temperature features -high temperatures and salinity stresses were well tolerated. Salinity tolerance was higher than 70%-80%, while high levels of salinity (5%) were well tolerated, even among strains with little or no mucus production (Figure 10). We assumed that a higher adaptation (possibily coadaptation) among these strains, the plants and other microbial populations, in the Caatinga semiarid environment, had some sort of influence on these results. We do not have the tools to affirm that, but we assume that what we Humans, may consider "extreme conditions", for the microbial biota, and maybe the leguminous trees, might be just the "normal"; at least, for these groups of isolates in this specific situation. We agree with Araújo & silva (2007) that, perhaps, a higher degree of microbial endemism, might be of quantitative importance regarding the presence of leguminous plants in the Caatinga. It may allow us to assert that the knowledge about the status of nodulation of native species in the Caatinga biome is somehow insufficient, which reinforce the need for subsequent studies with prospection of a broader spectrum of leguminous plants, allowing the discovery of new strains of bacteria able to perform natural nodulation, with subsequent application of agronomic efficiency, and potential strains tests for the identification of species that may be used for recovering degraded areas (Souza et al. 2013), and for agricultural e economic benefit.

Fig. 10. Increasing salinity concentration tolerance of rhizobia isolates (NaCl-1%, 3% and5%).



FINAL CONSIDERATIONS

We were able to identify several different survival strategies and responses among the selected isolates, especially regarding their specificity under local environmental conditions, resulting in a combination of auspicious results. We observed different responses regarding growth pace -fast and high-speed growth, both in the rainy and in the dry seasons; the influence of the soil, although we were not able to access the full survival strategy and response of the strains to this element. Besides, we observed that the selected strains were able to develop in a poor but relatively well-structured soil, with acidic pH values; under high temperatures (also demonstrating high average temperature tolerance in laboratory conditions); as well as water stress resistance -they survive in a semiarid environment, recurrently exposed to droughts. We believe that the presence of isolates that grew at a high-speed, in only one (1) day, may indicate a greater coadaptation, among the microbiota and the plants, to the semiarid conditions in the Caatinga. Semiarid areas present peculiar characteristics, with all its biodiversity under conditions of constant stress -either temperature, high salinity soils, low rainfall, etc.

There is a clue that the high phenotypic diversity observed in this study may be an indication of the ability of this bacterial group to maintain its functions under conditions that we consider of high stress. The conditions we consider stressful may be relative, even considering the inexistence of guarantees for the BNF fulfillment -since the occurrence of what we consider an "optimal situation" for microbial development and survival is not well-understood. It is worth to remember that our interest in BNF is often related to economic issues. For microorganims and plants to maintain their mutualistic relationship -in its full potential -many other factors may interact. Also, we need to assume that BNF may not be helpful nor necessary in many natural situations. We must learn to study the leguminous-rhizobia relationship in a holistic and less anthropogenic way.

The high level of tolerance to environmental stresses among the selected strains may be the result of adaptation processes along with the great diversity of native leguminous trees that reinforce the importance of studies in protected areas of semiarid regions in the Caatinga, as a way to ensure biodiversity protection and sustainable economic use of natural resources. The great phenotypic diversity of rhizobia found in this study may be an indication of a greater genetic biodiversity. It can also result from the stability of the soil, and a coadaptation response of isolates to local environmental conditions -independently of what we consider "natural stresses".

The fact that under natural conditions only one species was identified with root nodules –among all the eight leguminous trees -and limited to the rainy season; coupled with observations of no formation of root nodules in Mimosa acustistipula, and *Erythrina fusca* in the dry season, in greenhouse experiments –when bait plants died in early stages -may not be seen as a negative aspect. Instead, we assume that these results proved how closely associated many of these microsymbionts are with specific partners, and what intrinsic and extrinsic mechanisms would act to induce a selective or specific behavior and response.

More studies are needed to better understand the mechanism of response to climate and environmental stresses in the rhizobia-legume relation. Nevertheless, the production of nitrogen-fixing isolates from the semiarid Northeast of Brazil may have great potential.

Finally, we summarize the main results: (i) 92.42% of the isolates presented rapid growth; while, (ii) 52.24% presented acidification of the culture medium 79; regarding stresses tolerance, (iii) 84.93% of the isolates showed tolerance to high temperatures (45°C); (iv) 90.75% of the isolates were tolerant to high saline concentrations of NaCl (5%), indicating a high correlation between tolerance salinity and high temperatures.

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