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Agronomic efficiency of *Bradyrhizobium* in peanut under different environments in Brazilian Northeast

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Several legumes have natural ability to associate with nitrogen-fixing bacteria known as rhizobia. The efficiency of this association depends on the plant and bacterial genotype and the edaphoclimatic conditions. Peanut is a tropical legume able to associate with a wide range of rhizobia and the selection of efficient bacteria is important to increase the nitrogen fixation in this crop. In order to investigate the agronomic efficiency of two *Bradyrhizobium* strains, two peanut genotypes were used in field trails carried out in three environments located at Brazilian Northeast. The genotypes (BR1 and L7 Bege) were submitted to rhizobial inoculation (SEMIA 6144 or ESA 123, both *Bradyrhizobium* strains), and chemical nitrogen fertilization in randomized block design experiments. The following traits were analyzed: flowering (F), main axis height (MAH), number of nodules/plant (NN), number of pods/plant (NP) and weight of pods (WP). Differential responses were found in all to treatments to NN, NP and WP, in the three environments studied. Overall, ESA 123 showed good agronomic performance inducing higher pod production. The results support the evaluation of the *Bradyrhizobium* in further experiments aiming at its recommendation to commercial inoculants in Brazilian Northeast region.

Key words: Biological nitrogen fixation, inoculant, fertilization, symbiosis, rhizobia.

INTRODUCTION

Peanut (*Arachis hypogaea* L.) is a plant of South America, and considered as one of the main oilseeds grown in Brazil and worldwide. It is currently the fourth largest crop of oilseeds, being grown in more than 100 countries, with about 45 million tons, where 67% of world production is concentrated in China, India, Nigeria, United

States and Sudan (FAOSTAT, 2015; USDA, 2016). In Brazil, peanut crop is grown in different climates in Southeast, South and Northeast regions, by using robust cultivars in order to meet up with the food market (CONAB, 2015).

In Northeast region, peanut is cropped mainly by small

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farmers that adopt earliness and upright genotypes in agroecological systems. Peanut plants respond positively to nitrogen fertilization and to rhizobial inoculation (Melo, 2013). The N supply in plants is often provided by chemical fertilizers, some of them have low efficiency due to its natural transformations in soil (e.g. denitrification and volatilization), lead to losses that can achieve up to 70% of the N applied (Mortvedt et al., 1999; Signor and Cerri, 2013). Biological nitrogen fixation (BNF) is an alternative to nitrogen supply to several plants, especially legumes due to its association with rhizobia present in roots (Peix et al., 2015). In agriculture, this relationship could be exploited by production and use of inoculants containing efficient strains of rhizobia, and applied directly in seeds or soil (Nogueira and Hungria, 2013).

Peanut is a legume able to nodulate with a wide range of native rhizobia, which often is high competitive and low efficient to N fixation (Santos et al., 2007; Thies et al., 1991). This is why it is difficult to establish an effective association between peanut and rhizobia (Borges et al., 2007). Studies in this direction should be encouraged in order to identify high competitive and efficient strains to stimulate the use of inoculants in peanut, by growers. Currently, SEMIA 6144 is the unique Brazilian commercial *Bradyrhizobium* strain recommended to peanut, although several studies involving efficiency of strains collected from different environments have been done (Hoffman et al., 2007; Lyra et al., 2013; Torres-Júnior et al., 2014).

Rhizobia efficiency is dependent on several factors, such as soil fertility, weather and genotype x strain interaction (Marinho et al., 2014). The Brazilian Northeast has a large variation of soil and climate, and the predominant climate in the region is the dry and hot Semiarid, in which soils are often shallow and low fertile due to irregular water availability. These conditions are not favorable to proliferation of rhizobia inoculated in soil, although *Bradyrhizobium* has broad variability (Santos et al., 2005; Hoffman et al., 2007). Then, the identification and further recommendation of efficient rhizobia strains represent a wide benefit to peanut growers established in Northeast region.

Here, the agronomic efficiency of two peanut genotypes inoculated with two *Bradyrhizobium* strains in three different environments of Brazilian Northeast was evaluated.

MATERIALS AND METHODS

Genetic resources and rhizobia growth

Two bacterial strains were tested in the present study. The *Bradyrhizobium elkanii* SEMIA 6144 is used in commercial inoculants to peanut in Brazil. The isolate ESA 123 is a *Bradyrhizobium* sp. obtained from a trap-host experiment using soils from peanut production site in Barbalha, CE, in Brazilian Semiarid region. This bacterial isolate was previously characterized and selected by Cunha (2014).

Both bacterial isolates were grown in yeast extract-mannitol (YM)

liquid medium (Vincent, 1970) in constant stirring of 120 rpm during 6 days, up to the end of exponential growth phase. An aliquot of 50 mL of the bacterial broth were added to plastic bags containing 200 g of sterilized peat, mixed by hand carefully and stored at 10°C for further inoculation in seeds at sowing (7 days after the inoculant preparation).

Two earliness and upright peanut genotypes (BR1 and L7 Bege) were used in assays. BR 1 is a cultivar developed by Peanut Breeding Program, coordinated by Brazilian Company of Agricultural Research (Embrapa) and recommended to semiarid region (Santos et al., 2010) and L7 Bege is a top line, derived from a drought tolerant cultivar (Senegal 55 437), with broad adaptability to Northeast region (Vasconcelos et al., 2015).

Field trials

The trials were carried out in field, during rainy season of 2014, in three different environments in Northeast region (Table 1). Composite sample (6 sub-samples) of each soil was collected at 0-0.2 m depth to evaluate chemical characteristics according to Silva (2009) (Table 2). Based on the results, soils were corrected with 1.5 Mg ha⁻¹ of dolomitic limestone one month before the experiments set up. In addition, 60 kg ha⁻¹ of simple superphosphate and 30 kg ha⁻¹ of potassium chloride were used.

Each plot had five rows with 5 m, spaced in 0.7 m and data were collected from three central lines of each plot. Plants were spaced in 0.2 m. Three seed were sowed per hole, and further thinned to 2 plants per hole, at 15 days after emergence (DAE).

The treatments adopted were: a) inoculation of seeds with SEMIA 6144, b) inoculation of seeds with ESA 123, c) nitrogen fertilization (80 kg ha⁻¹ of ammonium sulphate splitted in two applications, at the sowing and 30 DAE) and d) absolute control (without inoculation or nitrogen fertilization). The inoculation was carried out soon before the sowing using 250 g of inoculants to 10 kg of seeds. The inoculants, seeds and 150 mL of a sticking agent (supersaturated sucrose solution, at 70% w/v), were putted in a plastic bag and mixed by hand. The inoculated seeds were dried at the shadow for 30 min prior to sowing.

A completely randomized blocks design was adopted, in a factorial scheme of 2 (plant genotypes) x 4 (inoculation treatments) x 3 (environments). At 88 DAE, the plant height were measured and further harvest. For the harvest, the roots were separated from the shoots and carefully washed in tap water and the nodules were detached and counted. The pods were separated from the plants, dried under the sun for 4 days, and then counted and weighted.

Five agronomical traits were evaluated: flowering (F), main axis height (MAH), number of nodules/plant (NN), number of pods/plant (NP) and weight of pods (WP). Data were submitted to analysis of variance using the Sisvar 5.3 software (Ferreira, 2009). Tukey test ($p < 0.05$) was adopted to mean comparisons, among genotypes (G), fertilization treatments (T), environments (E) and interaction effects.

RESULTS AND DISCUSSION

The treatments evaluated in this study induced to different responses in peanut genotypes to number of nodules/plant (NN), number of pods/plant (NP) and of weight of pods (WP), in all environments tested (Table 3). Effects of E x T and G x T interactions were also found meaning that the treatments promoted differences in agronomic traits of peanut genotypes grown in different sites. Flowering (F) and main axis height (MAH) were not influenced neither by treatments nor by environments

Table 1. Climatic characteristics of environments in Northeast region.

Site	Coordinates	C	RDC (mm)	T* (°C)	RH* (%)	Soil
Abreu e Lima, PE	07°54'43"S; 34°54'10"W, 19 m	T	1100	29	66	Neosol
Campina Grande, PB	07°13'50"S; 35°52'52"W, 551 m	S	409	22	84	Vertisol
Barbalha, CE	07°18'40"S; 39°18'15"W, 414 m	S	763	26	78	Vertisol

C- Climate: T - tropical, S – semiarid; RDC - total rainfall during peanut cycle; T - temperature; RH - relative humidity, *average during cycle.

Table 2. Chemical characteristics of the soil sampled at experimental fields before the experiments implementation.

Site	¹ pH	² OM g kg ⁻¹	³ mmol.c.dm ⁻³				⁴ P mg.dm ⁻³
			³ Al ⁺³	³ Ca ⁺²	³ Mg ⁺²	⁴ K ⁺	
Abreu e Lima, PE	6.6	13.8	nd ⁵	27.4	12.0	1.8	52.6
Campina Grande, PB	8.2	6.3	nd	46.1	8.4	3.2	72.3
Barbalha, CE	6.3	11.9	nd	67.9	30.9	2.2	12.1

¹pH in water (1:2.5); ²OM- organic matter (Walkley and Black method); ³extracted with KCl (1 mol.L⁻¹); ⁴Mehlich 1 method; ⁵not detected.

Table 3. Synthesis of variance analysis for peanut traits obtained from different treatments, in the three environments.

Variation source	DF	Mean Square				
		F	MAH	NN	NP	WP
Environment (E)	2	1718 ^{ns}	14051 ^{ns}	143703*	3516*	4183*
Genotype (G)	1	1.50 ^{ns}	63.42 ^{ns}	158559*	495*	3579*
Treatment (T)	3	2.18 ^{ns}	52.03 ^{ns}	2658.9*	92.22*	491*
E x T	6	0.37 ^{ns}	29.80 ^{ns}	4613 ^{ns}	35.98 ^{ns}	124*
G x T	3	0.63 ^{ns}	27.39 ^{ns}	8341 ^{ns}	12.26 ^{ns}	102*
G x E	3					
E x G x T	6	1.02 ^{ns}	17.56 ^{ns}	50.84 ^{ns}	30.03 ^{ns}	68.19 ^{ns}
E x T1	2	406*	3676.8*	12550.1 ^{ns}	691.6*	1281.5*
E x T2	2	427*	3756.3*	52628.9*	567.6*	258.6*
E x T3	2	435*	3374.6*	48693.1*	1270.3*	1516*
E x T4	2	450*	3332.9*	43672.3*	1095*	1501.4*
G x T1	1	0.66 ^{ns}	122.4*	56025.1*	87.7 ^{ns}	1225.9*
G x T2	1	0.04 ^{ns}	20.61 ^{ns}	4166.9 ^{ns}	51.6 ^{ns}	1293.3*
G x T3	1	2.66*	1.69 ^{ns}	71652.7*	169.2*	1148.4*
G x T4	1	0.04 ^{ns}	0.95 ^{ns}	51738.8*	223.5*	218.8*
Block	3					
Error	69					
Total	95					
Mean		24.85	45.50	253.61	22.13	40.38
CV (%)		2.5	10.9	29.5	22.7	18.1

DF - Degrees of freedom, CV - coefficient of variation, * statistically significant by F test ($p < 0.05$), ns - non significant, F - flowering (DAE), MAH - main axis height (cm), NN - number of nodules/plant, NP - number of pods/plant and WP - weight of pods (g), T1- No N-fertilization, T2- N-chemical, T3- ESA 123, T4- SEMIA 6144.

tested.

These traits show low variation in BR 1 and L7 Bege, both upright and earliness genotypes, with full pod

maturation between 85-90 days (Santos et al., 2013).

The means of treatments and E x G, E x T and G x T interactions are shown in Table 4. It was found that high

Table 4. Mean of isolate and interaction factors of traits in peanut grown under different treatments, in the three environments.

Environments (E)	F (DAE)	MAH (cm)	NN nod/plant	NP pods/plant	WP g/plant
E1- Abreu e Lima, PE	23.81	46.32	284.92 ^a	20.69 ^b	36.19 ^b
E2- Campina Grande, PB	25.47	44.96	299.24 ^a	21.13 ^b	38.07 ^b
E3- Barbalha, CE	25.15	45.23	176.67 ^b	25.10 ^a	46.87 ^a
Genotype (G)					
G1- BR 1	25.01	46.12	212.97 ^b	21.03 ^b	35.86 ^b
G2- L7 Bege	24.70	44.49	294.25 ^a	23.57 ^a	44.85 ^a
Treatment (T)					
T1- No N-fertilization	25.92	45.62	239.12 ^c	18.87 ^b	31.50 ^b
T2- N-chemical	23.86	47.14	253.06 ^b	21.55 ^{ab}	40.52 ^{ab}
T3- ESA 123	24.71	44.82	263.24 ^a	23.86 ^a	45.02 ^a
T4- SEMIA 6144	24.89	43.63	259.01 ^{ab}	23.75 ^a	44.42 ^a
Interactions					
Environments (E) x Genotypes (G)					
E1 x G1	24.62	43.13	218.05 ^{bc}	19.10 ^{bc}	34.97 ^c
E2 x G1	25.35	45.31	273.82 ^b	18.04 ^{bc}	37.99 ^b
E3 x G1	25.43	45.11	147.02 ^c	26.93 ^a	40.77 ^b
E1 x G2	23.00	47.52	351.78 ^a	22.28 ^b	38.87 ^b
E2 x G2	25.18	44.23	324.65 ^a	19.21 ^{bc}	41.96 ^b
E3 x G2	25.47	45.24	206.31 ^{bc}	27.20 ^a	47.68 ^a
Environments (E) x Treatments (T)					
E1 x T1	25.15	44.73	246.46 ^b	17.56 ^c	32.05 ^c
E2 x T1	25.00	45.49	274.54 ^{ab}	19.26 ^c	33.34 ^c
E3 x T1	26.00	45.06	196.35 ^c	18.73 ^c	36.12 ^{bc}
E1 x T2	25.50	44.39	305.80 ^a	21.44 ^b	38.06 ^{bc}
E2 x T2	25.12	44.82	293.72 ^{ab}	22.03 ^b	39.12 ^{bc}
E3 x T2	24.00	45.23	159.66 ^c	25.83 ^b	42.35 ^b
E1 x T3	23.25	46.15	309.46 ^a	22.74 ^b	44.20 ^b
E2 x T3	26.00	45.49	307.10 ^a	21.16 ^b	44.39 ^b
E3 x T3	26.05	46.84	173.17 ^c	36.35 ^a	50.33 ^a
E1 x T4	24.55	46.04	277.95 ^{ab}	21.03 ^b	40.35 ^{bc}
E2 x T4	24.25	45.58	321.58 ^a	25.05 ^b	43.14 ^b
E3 x T4	25.37	46.15	177.50 ^c	33.37 ^a	46.18 ^b
Genotypes (G) x Treatments (T)					
G1 x T1	25.08	45.88	190.80 ^d	16.94 ^c	33.85 ^c
G1 x T2	24.62	46.07	239.88 ^c	19.86 ^{bc}	35.98 ^c
G1 x T3	24.13	45.59	208.60 ^{cd}	20.77 ^{bc}	40.89 ^{ab}
G1 x T4	25.25	44.44	212.58 ^c	21.91 ^b	38.84 ^b
G2 x T1	25.05	44.37	287.43 ^b	21.76 ^b	37.75 ^b
G2 x T2	24.53	46.21	266.24 ^{bc}	22.88 ^b	43.96 ^a
G2 x T3	25.17	44.86	317.88 ^a	26.72 ^a	45.93 ^a
G2 x T4	25.33	44.83	305.44 ^a	25.96 ^a	42.86 ^{ab}

Means with the same letter in the column do not differ by the Tukey test ($p < 0.05$). F- flowering (DAE), MAH - main axis height (cm), NN- number of nodules/plant, NP- number of pods/plant, WP- weight of pods (g).

nodulation of rhizobia isolates at Abreu e Lima and Campina Grande, both benefited by warm weather in

these environments. In Barbalha, however, nodulation was expressively reduced possibly due to wide

adaptation of native rhizobia population to semiarid environment (E3 x T1), contributing to inhibition of occupation of the nodulation sites by SEMIA 6144 and ESA 123. Based on reports available in literature, nodulation in several legume-rhizobia systems are negatively influenced by high temperature and low soil moisture (Kahindi et al., 1997; Kulkarni et al., 2000), whose characteristics are often found in Brazilian Semiarid. However, some native isolates may overcome the unfavorable conditions and improve its nodulation capacity contributing to plant establishment and production (Hungria and Vargas, 2000; Marinho et al., 2014). It could explain the behavior of ESA 123, an isolated selected from Barbalha (Cunha, 2014), that contributed to increase the pod production of plants. This behavior was not found in SEMIA 6144 that was isolated from peanut production belt, in Southern region of Brazil.

Despite low nodulation seen in Barbalha, the number and weight of pods were higher than in others environments, especially in ESA 123 treatment (E3 x T3), indicating that even with climatic limitations, this isolate was more responsive to improve the production of both peanut genotypes (G1 x T3 and G2 x T3).

Taking in account the behavior of genotypes as to nodulation, it was found that L7 Bege was more beneficial to rhizobia inoculation, especially with ESA 123, an isolate also adapted to semiarid region. This top line is a Valencia-high yield, obtained by crossing with a Brazilian high yield (IAC Tupã) and an African drought resistant (Senegal 55437) cultivar. As seen in Table 4, the nodulation, number of pods and weight of pods were higher in L7 Bege than BR 1, in all environments.

Although, the results obtained with BR 1 have been less expressive than those with L7 Bege, promising results were also achieved, strengthening the benefits of rhizobial inoculation in management of this cultivar. The inoculation with SEMIA 6144 achieved an increasing of 8.0 and 14.7%, in relation to nitrogen fertilization and control, respectively. With ESA 123, the rates were 13.6 and 20.8%, respectively. In others studies, positive responses of BR1 to inoculation with SEMIA 6144 were found in pot experiments (Melo, 2013; Torres-Júnior et al., 2014), but the performance of this association were not evaluated, up to now, for field conditions. These results are quite relevant because a positive interaction of BR 1 with a recommended strain is very important for the spread of adoption of inoculation practice by peanut farmers.

The performance seen here with ESA 123 in both peanut genotypes and in three environments was very satisfactory because it indicates the potential of this strain for further use in others assays, aiming at recommendation of commercial inoculants. In Brazil, the selection of new rhizobia to legume crops is carried out under the determinations of the Ministry of Agriculture, Livestock and Food Supply (MAPA, 2011). The stability of agronomic efficiency in different environments is one of

the most important criteria in selection procedures. Studies based on selection of new *Bradyrhizobium* strains to commercial legumes have been carried out in Brazilian Northeast region, in last years (Marinho et al., 2014). Even so, there is still lack of knowledge regarding strain selection for peanut, and this work offers broad information on the behavior of upright genotypes evaluated under inoculation of a new *Bradyrhizobium* strain in different environments of Brazilian Northeast region.

Conclusions

The strain ESA 123 showed agronomic benefits of peanut in different environments of Brazilian Northeast region. The results indicate ESA 123 for standardized experiments in different locations aiming further at recommendation of commercial inoculants of peanut in Brazil.

Conflict of interest

The authors have not declared any conflict of interest.

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