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Response of traditional upland rice varieties to inoculation with selected diazotrophic bacteria isolated from rice cropped at the Northeast region of Brazil

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ABSTRACT

The largest numbers of the Brazilian traditional upland rice varieties are found in the Maranhão state, Northeast region of Brazil. However, no information is available on the diazotrophic bacterial population associated as well as the plant growth promoting potential when these traditional genotypes are inoculated with native strains. Here, we evaluated the response of ten traditional rice varieties to inoculation with ten diazotrophic strains, previously isolated from rice soil of this region and screened for their ability to produce indole-3-acetic acid (IAA) *in vitro*. The procedure for selection of the best diazotrophic strain/rice variety interaction involved three steps: gnotobiotic conditions, soil pot and field experiments. The gnotobiotic experiment showed that the *Azospirillum amazonense* strain AR3122 increased the biomass of the traditional varieties Cana Roxa and Cana Forte (28 and 48%, respectively) while this effect was less evident for the other combination of strains/rice varieties. The soil pot experiment showed that the combination of *Burkholderia vietnamiensis* strain AR 1122 and traditional variety Arroz 70 was superior to the other strains/varieties and the treatment fertilized with 100 kg N ha^{-1}. The best performance of the *Burkholderia vietnamiensis* strain AR1122/variety Arroz 70 was confirmed in the field experiment. There was an increase of up 10 and 29% in the grain yield in comparison to both the N fertilization and *Herbaspirillum seropedicae* ZAE 94 strain treatments, respectively. In contrast, the response of the commercial variety Bonança to inoculation with strain AR1122 was much lower, suggesting that a biofertilizer inoculation program for traditional rice varieties should consider the genetic interaction between strain and rice variety. The diazotrophic *B. vietmaniensis* strain AR1122 was a good biofertilizer candidate for inoculation of traditional rice varieties and therefore should be used for further studies to confirm the strain-genotype effect envisaging a sustainable rice crop system mainly in the Northeast region of Brazil.

1. Introduction

Rice (*Oryza sativa L.*) is an excellent source of carbohydrates and proteins and has been considered by FAO a strategic crop for food security of the world population due to its ample adaptation to climates and soils (FAO, 2006). It is well known the need for increasing global rice production to attend the food demand mainly of highly populated countries like China and India. It has been estimated that the global rice production must reach the equivalent to 430 Million tons by the year 2030 (Timmer et al., 2010) and about 455 million of tons by the year 2050 (Mohanty et al., 2010). It is also expected an increase in the amount of chemical fertilizers to be applied (Gregory et al., 2010); including nitrogen (N) that is the most limiting nutrient for the rice crop (Ladha and Reddy, 2003). The literature has shown that only one- third of the applied N is utilized by rice plants while the other one-third remains in the soil at the crop harvest and the rest is lost as gas to the atmosphere, mostly through ammonia volatilization (Buresh et al., 2008). Thus, it becomes important to find alternatives to reduce the use of N fertilizers applied to rice crop without decreasing the productivity and causing risks of environmental pollution.

The association of nitrogen-fixing bacteria or diazotrophs with rice varieties is one alternative that has been strategically thought to replace part of the N fertilizer required by the plant and in addition, indirectly helping the plant to assess other nutrients added or naturally present in the soil. Many rhizospheric and endophytic diazotrophic species, including *Azospirillum brasilense,*

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Table 1
Origin and indole-3-acetic acid production (IAA) by the bacterial isolates belonging to the genera *Azospirillum, Burkholderia* and *Sphingomonas* utilized in association with traditional rice cultivar from Maranhão (Ma) state to select strains with potential biofertilizer use.

Strain identification	Bacterial Species	Locality of soil sampled	Part of the plant[a]	IAA production ((M mL^{-1})
AR3122	*Azospirillum amazonense*	Arari, Ma	RD	186
VR218	*Azospirillum amazonense*	Vitória do Mearim, Ma	RD	303
VF2213	*Azospirillum amazonense*	Vitória do Mearim, Ma	PA	164
AR2112	*Sphingomonas* sp.	Arari, Ma	RD	219
BF1358	*Burkholderia vietnamiensis*	Bacabal, Ma	PA	75
AR1135	*Burkholderia* sp.	Arari, Ma	RL	36
BR2113	*Burkholderia vietnamiensis*	Bacabal, Ma	RD	16
AR1124	*Burkholderia vietnamiensis*	Arari, Ma	RD	52
VR2117	*Burkholderia vietnamiensis*	Vitória do Mearim, Ma	RL	32
AR1122	*Burkholderia vietnamiensis*	Arari, Ma	RL	62
ZAE94–reference biofertilizer strain	*Herbaspirillum seropedicae*	Seropédica, RJ	RD	88

RL – washed roots; RD – disinfested roots; PA – aerial part.

Azospirillum amazonense, Herbaspirillum seropedicae, Burkholderia tropica, Burkholderia vietnamiensis and *Sphigomonas* sp., have been found colonizing in high numbers many rice varieties growing in tropical regions (Baldani and Baldani, 2005). It has been demonstrated that certain rice varieties respond positively to inoculation when selected diazotrophic strains from these species were used as biofertilizer (Tran Van et al., 2000; Govindarajan et al., 2008; Rodrigues et al., 2008; Ferreira et al., 2010). In addition, ^{15}N analysis of inoculated rice varieties indicated that part of the N accumulated in plant tissues and grains is derived from the biological nitrogen fixation (BNF) process (Baldani et al., 2000; Boddey et al., 1995; Malarvizhi and Ladha, 1999). The literature has also shown that in addition to BNF, some of these inoculated diazotrophic bacteria may also enhance the plant nutrition by using other mechanisms such as production of phytohormones (Peng et al., 2002), siderophore production (Govindarajan et al., 2008) and solubilization of P and Zn (Saravanan et al., 2008). Therefore, selection of efficient strains to enhance yield productivity of rice should consider the characteristics above as well as its ability to establish in the roots and compete with the native microbial community.

The plant genotype, besides environmental factors, is a very important agronomical aspect that should be considered during selection of diazotrophic strains since it may act as selective filter for the rhizospheric and endophytic bacteria that associate with the plants. Recent study carried out with 10 rice cultivars strongly suggested that plant genotype determined the composition of the different bacterial communities across cultivars (Hardoim et al., 2011). The authors observed that traditional and modern rice cultivars are constituted by different bacterial association and suggested that part of this effect may due to the plant breeding strategies for higher-yield crops. In fact, previous studies have already shown that traditional rice varieties harbor bacterial populations different from those found in modern varieties (Engelhard et al., 2000; Knauth et al., 2005). In addition, few reports have also demonstrated the crucial role played by genotypes in the population of diazotrophic endophytic bacteria colonizing rice plant tissues (Elbeltagy et al., 2001; Baldani et al., 2000; Sasaki et al., 2010).

In the Maranhão state, northeast region of Brazil, the largest numbers of traditional Brazilian upland varieties of rice are found (Fonseca et al., 1982). These genotypes are less dependent on N fertilizer and are more adapted to the region since no or low N fertilizer level has been applied along the years due to the traditional practice implemented by local farmers (Ferraz Junior et al., 1997). Therefore, these traditional varieties may have acted as a "selective filter" on the diazotrophic bacterial population associated so that only more efficient genotype/bacteria interactions could have been established along the years. So far, no study was carried out on the diazotrophic bacterial population associated with these traditional rice varieties.

The objective of this study was to evaluate the response of traditional rice varieties, grown in a rice crop region of Maranhão state, to inoculation with native diazotrophic bacteria isolated from soil of the same region envisaging the selection of strains with higher growth promoting potential than strain *H. seropedicae* ZAE94, commonly used as biofertilizer in commercial rice varieties cultivated in Brazil.

2. Materials and methods

2.1. Strain isolation and physiological and molecular characterization

The strains were isolated from different plant tissues (roots, culms and leaves) of the traditional rice varieties Zebu Branco and Manteiga grown in soil collected from three traditional rice crop regions (Arari, Bacabal and Vitória do Mearim) of Maranhão state, Northeast of Brazil. These two and the other traditional rice varieties have been cultivated for many years by the local farmers that traditionally apply very low amount of fertilizers including N. A collection of 228 isolates were obtained from these rice varieties and evaluated for their ability to reduce acetylene in semi-solid media (NFb, JNFb, JMV and LGI) and production of IAA. Ten (10) diazotrophic isolates (checked by acetylene reduction activity) were selected based on their high, medium and low abilities to produce IAA (Table 1). These isolates were further identified based on the almost complete (approximately 1400 bp) 16S rRNA sequences according to the protocol described by Videira et al. (2009) and taxonomically clustered in the genera *Azospirillum*, *Sphingomonas* and *Burkholderia* (Table 1). The strain *H. seropedicae* ZAE94 was used as a reference biofertilizer inoculant considering its positive effect on commercial rice varieties (Baldani et al., 2000; Guimarães et al., 2003).

2.2. Gnotobiotic experiment

The experiment was carried in a complete randomized design with a factorial 11 × 12 (11 genotypes and 12 inoculation treatments) and four replicates. Ten strains were used: six (06) of the genus *Burkholderia* (VR2117, AR1122, AR1124, BR2113, AR1135 and BF1358), three (03) of *A. amazonense* (VR218, VF2213 and AR3122) and one (01) *Sphingomonas* sp (AR2112). The strain ZAE 94 was used as positive inoculant control while autoclaved cells were applied in the absolute control. Ten traditional varieties of rice developed and cultivated for many decades in a rice crop region of Maranhão state were tested: five (05) with high (>8%) crude protein content (Zebu Branco, Arroz 70, Cana Roxa, Bacabinha and Braquiaria) and five (05) with medium/low (6–8%) crude protein content (Cana forte, Come Cru, Pingo D'água, Lajeado Liso and