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Inoculation of paddy rice with *Azospirillum brasilense* and *Pseudomonas fluorescens*: Impact of plant genotypes on rhizosphere microbial communities and field crop production

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ABSTRACT

Rice is the third largest global food crop. Traditional practice to achieve maximum yields of rice is associated with the availability of mineral nitrogen and fertilization. This can lead to pollution of waterways. This can be particularly important in paddy rice production in north-eastern Argentina. Biofertilization or inoculation with plant growth-promoting bacteria (PGPB) is a sustainable alternative for agro-ecosystems. Inoculation of wheat, maize, and soybean is a widespread agricultural practice that has proved to be efficient in increasing production and promoting nutrition of these crops. This work measures the response of three rice cultivars to PGPB inoculation under field conditions with a commercial formulation containing strains of *Pseudomonas fluorescens* and *Azospirillum brasilense*. The experiment was performed in a farm plot located near Villa Clara, Entre Ríos. A factorial complete block design with four replicates was applied. Samples were taken at tillering and physiological maturity. Aerial biomass, grain yield, and its components were determined. Culturable microorganisms were analyzed in rhizosphere samples. Counts of most probable number of microaerophilic, nitrogen-fixing microorganisms and community-level physiological profiles of carbon-source utilization were evaluated at physiological maturity. Also, DNA extraction, *nif*H gene amplification, and terminal restriction fragment length polymorphism (T-RFLP) analysis were performed to analyze molecular diversity of diazotrophic communities associated with rice roots. Data showed differences between rice genotypes. Inoculation with PGPB did not have significant impact on culturable microbial communities and patterns of T-RFLP. Some fragments obtained by restriction with enzymes *Hae*III and *Hha*I differentiated between inoculation treatments and rice genotypes. PGPB inoculation increased aerial biomass production, harvest index, and grain yield of the Supremo 13 cultivar by 4.7%, 16%, and 20.2%, respectively. Inoculation of the Yeruá cultivar increased aerial biomass by 1.9% and grain yield by 11%. On the other hand, control plants of the Cambá INTA cultivar produced 8.7% and 7.3% more aerial biomass and grain yield than inoculated plants, respectively. Inoculation reduced the percentage of chaffy grains of the three rice cultivars. The results indicate that the combined inoculation with *P. fluorescens* and *A. brasilense* has significant potential when applied to rice.

1. Introduction

Rice, wheat and maize provide two thirds of the energy in human diets. At current population growth rates, worldwide production will have to increase by 40% by 2020 (Vallejo et al., 2008). As a result of intensifying agricultural production, including fertilizers, pesticides, irrigation, genetic improvements, and double cropping, yields per surface unit and time has increased in the past two decades. However, future possibilities of increasing production are restricted by the limited additional suitable cropland. Thus, the increase of productivity is probably the only way to fulfill food demands. Increasing production without harming the environment is a challenge that requires improvement of soil quality and precise handling of all agricultural practices (Cassman, 1999; Naiman et al., 2009).

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Rice exhibits wide adaptability to different environments, which makes it the most widespread crop in the world. Breeding rice by selecting specific characteristics can adapt the crop to different areas with dissimilar levels of environmental stress. It can grow under drought conditions, shallow flooding, and water up to 50 cm of water (Catling, 1992). It is cultivated in a wide range of latitudes, as well as up to 3000 m elevation. In north-eastern Argentina, rice crops are grown under flood irrigation with few events of extreme temperatures (Arguissain, 2006).

Negative impacts of agriculture on the environment and high costs of production has led to initiatives to develop sustainable agriculture, production systems that provide reasonable economic returns to farmers and rural communities without affecting productive resources, preserving environmental quality, and creating safe and healthy food in sufficient quantities to meet the demands of growing populations (Benbrook, 1999).

Part of the strategy of sustainable agriculture is to find technologies that increase yields and reduce the use of chemical fertilizers (García de Salamone et al., 2010). One alternative is plant growth-promoting bacteria (PGPB) applied as inoculants (Bonilla et al., 2000). Among the PGPB, strains of *Azospirillum* are one of most studied genera. They can increase growth, development, and yield of a large number of plant species (Bashan et al., 2004; Cassán and García de Salamone, 2008). Numerous strains have been isolated from the root surface of many plants, including maize, wheat, rice, sorghum, oats, and forage grasses (Caballero-Mellado, 2007). *Azospirillum* spp. can produce and metabolize plant growth regulators as one of the main mechanisms to promote plant growth and development in inoculated plants (Okon and Labandera-Gonzalez, 1994). Biological nitrogen fixation is another mechanism in which these PGPB are involved (Baldani and Baldani, 2005; García de Salamone and Döbereiner, 1996). Importantly, grasses are capable of establishing symbiosis with diazotrophic bacteria, such as the genus *Azospirillum*. Nitrogen is the major limiting nutrient for rice production and so, bacterial contributions of nitrogen to plants could be vital at critical stages of plant development, such as reproduction and generation of tillers (Velazco, 2001). Physiological changes in rice and wheat inoculated with *Azospirillum* could favor biological nitrogen fixation and modify activity and number of microorganisms associated with the nitrogen cycle (García de Salamone et al., 2009). In further work, the bacterial strains responsible for efficiently supplying high levels of nitrogen through biological nitrogen fixation were identified (García de Salamone et al., 2010). Although numerous studies have shown the benefits of inoculation with *Azospirillum* on growth and production of different crops, the use of these PGPB is not widespread in rice production. It is known that plant-PGPB interactions can occur at different levels, influenced by genotype-environment interactions in response to inoculation (García de Salamone and Döbereiner, 1996; Olivares et al., 1996; Azevedo et al., 2005). The interaction of genotype-environment-inoculant points out the need for studies to increase the efficiency of inoculation (García de Salamone and Monzón de Asconegui, 2008).

Maintaining soil fertility is essential for sustainable production of grain and soil microorganisms play a fundamental role. The benefits of microbial inoculation on soil fertility have been documented in many studies (Jeffries, 1985; Kaushik, 1985; Roger et al., 1993; Gaur, 1990).

PGPB as biocontrol agents of diseases has also emerged as a tool in sustainable management programs. *Pseudomonas fluorescens* controls diseases, such as rice stem rot (*Sclerotium oryzae*) and rice sheath rot (*Rhizoctonia* spp.), under *in vitro* and under field conditions in north-eastern and north-western Argentina (Pedraza et al., 2009). This PGPB produces increased yields in wheat (Naiman et al., 2009) and produces high levels of cytokinins (García de Salamone et al., 2001). Initially, monoxenic inoculants have mainly been used.

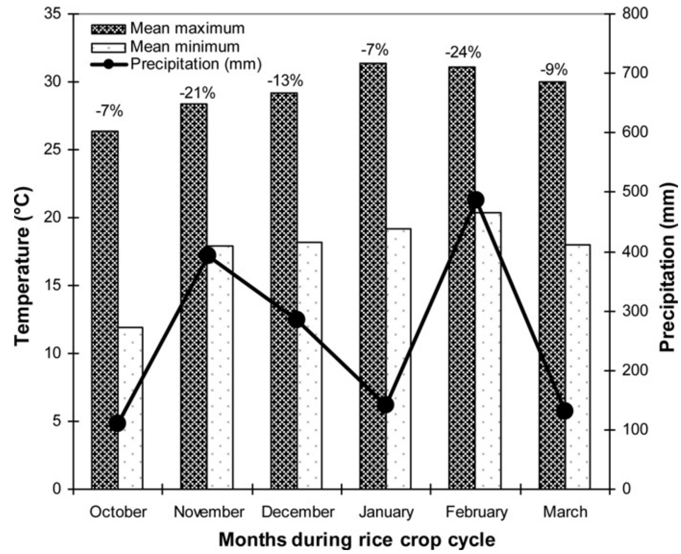

Fig. 1. Monthly precipitation, mean monthly maximum and minimum temperature, and relative differences in solar radiation during the rice crop cycle. Percentages for each month indicate relative differences in solar radiation with respect to average historical values at the site.

In recent years, there have been attempts to combine *Azospirillum brasilense* and *P. fluorescens*.

This study was planned to test the hypothesis that combined inoculation of PGPBs can modify rhizosphere microbial communities and growth and yield of rice grown under field conditions. We evaluated the response to a commercial liquid microbial inoculant containing *A. brasilense* and *P. fluorescens* to promote growth and yield of three rice cultivars under field conditions in Argentina. Additionally, we analyzed the effects of this mixed microbial inoculant and rice cultivars on the structure and physiology of microbial communities associated with rice root system.

2. Materials and methods

2.1. Field site and climate conditions

The experiments were conducted on farmland (31°44′25.4″S, 58°46′12.2″W) near Villa Clara in the Department of Villaguay in the Province of Entre Ríos. This region experiences warm and humid weather with an average temperature ranging between 17 and 20 °C and average annual rainfall of 1200 mm. Fig. 1 shows monthly maximum and minimum temperatures and rainfall during the field trials. The landscape of the experimental area mostly consisted of gently sloping alluvial plain. Fine textured clayed soils, mostly consisting of montmorillonite dominated in the experimental plots, thus hampering free movement of water through the soil profile. Chemical characteristics of the upper soil layer (30 cm) just before sowing were: pH (7.5 of 1:2.5 soil:water), electrical conductance (2.5 dS m^{-1}), total organic matter (3.1%), total N (0.16%), and available P (12 mg kg^{-1}).

2.2. Rice cultivars

We used certified seeds of the cultivars Cambá INTA, Supremo 13, and Yeruá supplied by the San Salvador Rice Coop of San Salvador, Entre Ríos, Argentina. Characteristics of the cultivars were described by Livore (2006); this is summarized in Table 1.