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Feasibility of transference of inoculation-related technologies: A case study of evaluation of soybean rhizobial strains under the agro-climatic conditions of Brazil and Mozambique

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ABSTRACT

The soybean-*Bradyrhizobium* symbiosis can be very effective in fixing nitrogen and supply nearly all plant's demand on this nutrient, obviating the need for N-fertilizers. Brazil has been investing in research and use of inoculants for soybean for decades and with the expansion of the crop in African countries, the feasibility of transference of biological nitrogen fixation (BNF) technologies between the continents should be investigated. We evaluated the performance of five strains (four Brazilian and one North American) in the 2013/2014 and 2014/2015 crop seasons in Brazil (four sites) and Mozambique (five sites). The experimental areas were located in relatively similar agro-climatic regions and had soybean nodulating rhizobial population ranging from $\ll 10$ to 2×10^5 cells g^{-1} soil. The treatments were: (1) NI, non-inoculated control with no N-fertilizer; (2) NI + N, non-inoculated control with 200 kg of N ha^{-1}; and inoculated with (3) *Bradyrhizobium japonicum* SEMIA 5079; (4) *B. diazoefficiens* SEMIA 5080; (5) *B. elkanii* SEMIA 587; (6) *B. elkanii* SEMIA 5019; (7) *B. diazoefficiens* USDA 110; (8) SEMIA 5079 + 5080 (only tested in Brazil). The best inoculation treatments across locations and crop seasons in Brazil were SEMIA 5079 + 5080, SEMIA 5079 and USDA 110, with average grain yield gains of 4–5% in relation to the non-inoculated treatment. SEMIA 5079, SEMIA 5080, SEMIA 5019 and USDA 110 were the best strains in Mozambique, with average 20–29% grain yield gains over the non-inoculated treatment. Moreover, the four best performing strains in Mozambique resulted in similar or better yields than the non-inoculated + N treatment, confirming the BNF as an alternative to N-fertilizers. The results also confirm the feasibility to transfer soybean inoculation technologies between countries, speeding up the establishment of sustainable cropping systems.

1. Introduction

Soybean [*Glycine max* (L.) Merrill] has potential to play a major role in responding to global food insecurity that results from mounting demographic pressures. The world population is projected to grow beyond 10 billion by 2100 (Gerland et al., 2014), and much of the increase will occur in Africa (Cleland, 2013), where hunger is already a threat. With high concentration of seed protein (40%), that provides all essential amino acids in sufficient amounts for human health, and high seed oil content (20%), soybean has many uses, encompassing human food, animal feed and biofuels. Moreover, soybean offers a number of advantages in sustainable cropping systems, including the ability to symbiotically fix atmospheric nitrogen (N_2), which obviates the reliance on N-fertilizers.

Numerous reports testify that when soybean is grown for the first time in new areas outside Southeast Asia, its centre of origin and domestication, it generally requires inoculation with exotic strains (Pulver et al., 1985; Hungria et al., 2006b; Abaidoo et al., 2007; Giller et al., 2011; Hungria and Mendes, 2015). In Africa, where the distribution of inoculants represents another limitation, a strategy consisting in the use of promiscuous soybean genotypes—capable of forming nodules with indigenous rhizobia (Pulver et al., 1985; Abaidoo et al., 2007; Tefera, 2011)—has been used for decades; this strategy would be useful especially for smallholder farmers with no access to inoculants (Mpepereki

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et al., 2000). Nevertheless, with cropping intensification, the search for soybean genotypes with higher yield potential but requiring inoculation is scaling up.

Soybean response to inoculation is dependent on a number of environmental factors including soil N availability (Thies et al., 1991; Singleton et al., 1992), temperature (Hungria and Vargas, 2000), pH (Giller, 2001; Al-Falih, 2002), salinity (Zahran, 2010), P availability (Ronner et al., 2016) and, more importantly, indigenous rhizobial populations (Thies et al., 1992; Osunde et al., 2003). Very often, elite inoculant strains fail to overcome the competition barrier for nodule occupancy imposed by indigenous or naturalized rhizobia (Thies et al., 1992; Streeter, 1994; Vlassak et al., 1997; Al-Falih, 2002), most times ineffective but very competitive and already adapted to the environment (Streeter, 1994; Al-Falih, 2002; Grönemeyer et al., 2014). However, strong evidence of inoculation success in areas with high rhizobial population, of 10^3–10^6 cells g^{-1} of soil, has been published from Brazil (Hungria et al., 2005, 2006a, 2013; Campo et al., 2009; Hungria and Mendes, 2015), opening a window for inoculation research in other geographic regions.

Ecological studies on rhizobia have established that exogenous inoculant strains undergo genetic changes (Schloter et al., 2000; Barcellos et al., 2007) and may acquire superior competitive abilities as they become naturalized (Dunigan et al., 1984; Dowdle and Bohlool, 1987; Hungria and Mendes, 2015). The success of inoculation and nitrogen fixation on soybean in Brazil is chiefly ascribed to strain selection programs that took place for over half a century, in addition to the development of proper inoculation methods (Hungria et al., 2006a; Hungria and Mendes, 2015). On the contrary, in Mozambique soybean is a relatively new crop practiced primarily with promiscuous varieties without inoculation (Gyogluu et al., 2016). In recent years, nevertheless, the increased demand for soybean grain to supply the chicken industry and for export (Dias and Amane, 2011) has led to search for more productive non-promiscuous genotypes, which are generally responsive to commercial inoculants. The agro-climatic conditions of the soybean production areas in Mozambique are similar to the major soybean growing areas in Brazil, raising the question on whether the inoculant strains that perform well on a variety of agro-climatic zones in Brazil could be successfully transferred to Mozambique, saving time, labour and money.

The objective of this study was to compare the performance of four elite *Bradyrhizobium* strains from Brazil (SEMIA 587, 5019, 5079, and 5080) and another strain adopted as standard inoculant in many African countries (USDA 110) in trials carried out with non-promiscuous soybean genotypes in Brazil (four sites) and Mozambique (five sites).

2. Material and methods

2.1. Sites description: location, climate and soil characterization

Climate and soil classification (Table 1), soil chemical properties and rhizobial counts (Table 2), rainfall (Supplementary Table 1) and temperature (Supplementary Table 2) data are presented on the indicated tables. Sixty days prior to commencing the experiments, 20 soil sub-samples (0–20 cm) were collected at each site to evaluate biological, physical and chemical properties. Rhizobial population sizes were estimated by the most probable number (MPN) method (Vincent, 1970) with soybean cultivar BMX Potência RR (in Brazil) or Storm (in Mozambique). Silt, sand and clay fractions were determined by the hydrometer method (Kilmer and Alexander, 1949). In Mozambique, soil pH was determined in H_2O (1/2; soil/water) 60 min after agitation. Ca, Mg, Al, K and P were determined by inductively coupled plasma optical emission spectroscopy (ICP-OES) after extraction with Mehlich-3 (Sims, 1989). In Brazil, chemical analyses were performed as described by Sparks et al. (1996). Soil pH was determined in 0.01 mol L^{-1} $CaCl_2$ (1/2.5; soil/solution). Exchangeable Al, Mg and Ca were extracted with 1 mol L^{-1} KCl (1:10; soil/solution) after agitation for 10 min, P and K were extracted with Mehlich-1 after 10 min agitation. Aluminum was determined by titration with 0.015 mol L^{-1} standardized NaOH with indicator bromothymol blue, K was determined in a flame photometer, Ca and Mg were determined in an atomic absorption spectrophotometer, and P by the molybdenum-blue method with $C_6H_8O_6$ as reducing agent. In both countries soil organic carbon (SC) was determined by the Walkley-Black chromic acid wet oxidation method (Walkley and Black, 1934) and soil organic matter (SOM) was obtained considering SOM = 1.724 × SC.

In Mozambique, all trials were established in areas with no previous soybean cropping history or rhizobial inoculation, whereas in Brazil, the experiments were conducted in areas with or without soybean cultivation history. In Brazil, based on the results of the soil analyses, where applicable, lime was applied to rise bases saturation to 70% (southeast region) or 50% (central region).

2.2. Treatments and trials management

Thirty days before sowing, the areas were weeded with 2.5 L ha^{-1} of glyphosate ($C_3H_8NO_5P$) (in Brazil only). The experiments consisted of the following treatments, (1) NI, non-inoculated and non-N-fertilized control (symbiosis relied on indigenous or naturalized rhizobial populations); (2) NI + N, non-inoculated control with 200 kg of N ha^{-1} as urea (CH_4N_2O, 46.6%N), applied 50% at sowing and 50% at R2

Table 1
Location, climate, soil type and textural class of the experimental sites.

Experimental site	Georeference			Climate[1]	Soil type[2]	Textural class[3]
	Latitude	Longitude	Altitude (m)			
Brazil						
Londrina	23°11′S	51°11′W	620	*Cfa*	Rhodic Ferralsols	Clay
Maracaí	22°36′S	50°40′W	475	*Cfa*	Ferric Luvisols	Sandy
Ponta Grossa	25°13′S	50°01′W	880	*Cfb*	Orthic Ferralsols	Sandy clay loamy
Rio Verde	17°47′S	50°54′W	730	*Aw*	Acric Ferralsols	Sandy clay
Mozambique						
Muriaze	15°16′S	39°19′E	363	*Aw*	Ferric Luvisols	Sandy clay loamy
Nkhame	14°38′S	33°59′E	1115	*Cwa*	Orthic Ferralsols	Sandy loamy
Ntengo	14°33′S	34°11′E	1225	*Cwa*	Orthic Ferralsols	Clay
Ruace	15°08′S	36°25′E	673	*Cwa*	Rhodic Ferralsols	Sandy
Sussundenga	19°19′S	33°15′E	611	Cwa	Rhodic Ferralsols	Sandy

[1] Based on Köppen-Geiger climate classification (Pidwirny, 2011).
[2] Based on FAO soil classification (FAO, 2016).
[3] Based on USDA textural soil classification (USDA, 1987).