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Strategies to promote early nodulation in soybean under drought

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ABSTRACT

Biological nitrogen fixation (BNF) is a sustainable process that dismisses the use of supplemental N-fertilizers in soybean [*Glycine max* (L.) Merrill]. Strategies to provide early nodulation may increase the effectiveness of BNF under stressing conditions like drought. We assessed the effects of inoculation of *Bradyhizobium*, co-inoculation of *Bradyrhizobium* and *Azospirillum*, and addition of microbial secondary metabolites (MSM) on nodulation parameters and soybean yield in four field experiments in two growing seasons, 2013/14 and 2014/15, in Southern Brazil. The treatments were: non-inoculated (Ni) control; Ni + N-fertilizer (100 kg ha^{-1} at sowing and 100 kg ha^{-1} at full flowering, as urea); Inoculated with *Bradyrhizobium* (I); Co-inoculated with *Bradyrhizobium* + *Azospirillum brasilense* (Co-I); Co-I + microbial secondary metabolites (MSM) and I + MSM. All trials were rainfed and the second trial in 2014/15 was severely affected by drought and high temperatures. The co-inoculation with *Azospirillum* increased the soybean nodulation at early developmental stages and resulted in higher shoot N accumulation and plant growth, especially under drought. The addition of MSM attenuated the effect of drought on nodulation and in one trial increased the grain yield by 15% and 7% in relation to the N-fertilizer and sole inoculation with *Bradyrhizobium*, respectively. The strategy of co-inoculation stimulates an early nodulation and helps the maintenance of nodulation under drought; moreover, the addition of MSM improves nodulation and may increase the grain yield.

1. Introduction

Soybean [*Glycine max* (L.) Merrill] is the most widely grown oilseed worldwide, having reached a global 318.25 million metric ton in 2014/15 crop season (USDA, 2015). Currently, Brazil is the second largest producer, with 96.04 million metric ton in 2014/15 (CONAB, 2015). The success of the soybean crop in Brazil is in part attributed to the symbiosis with *Bradyrhizobium* and the biological nitrogen fixation (BNF) process, which provides nearly all N required and ensures high yields without supplemental mineral N (Alves et al., 2003; Hungria et al., 2006a).

However, drought and high temperatures (Hungria and Vargas, 2000; Sinclair et al., 2007; Chalk et al., 2010), or incompatibility of the microsymbiont with chemicals in the seed treatment (Hungria et al., 2015) may impair the BNF effectiveness. This biological process is negatively affected by drought even before the transpiration rate and photosynthesis (King and Purcell, 2005; Sinclair et al., 2007; Arrese-Igor et al., 2011). The impact of drought on plant and microsymbiont depends on the intensity, duration, and the plant developmental stage (Christophe et al., 2011). More drastic effects on nodulation and grain yield generally occur during the establishment of the symbiosis and formation of nodules (V2), full flowering (R2), or grain filling (R5) (Streeter, 2003; González-Dugo et al., 2010; Christophe et al., 2011).

Strategies to stimulate the early establishment of the symbiosis may result in increases in nodulation, nodule occupancy and more effective BNF, which might result in more even yields under drought (Hungria et al., 2006a; Chibeba et al., 2015). The co-inoculation of *Bradyrhizobium* with plant growth-promoting rhizobacteria like *Azospirillum* has been a beneficial strategy to promote early nodulation, BNF, and improve the crop's performance

Abbreviations: BNF, biological nitrogen fixation; CFU, colony forming units; DAE, days after emergence; LCO, lipo-chitooligosaccharides; NDVI, normalized difference vegetation index; PET, potential evapotranspiration; MSM, microbial secondary metabolites.

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and grain yield (Hungria et al., 2013; Chibeba et al., 2015). These results are attributed to the ability of plant growth-promoting rhizobacteria to produce phytohormones that stimulate the root system for exploring the surrounding soil (Saharan and Nehra, 2011), and may increase tolerance to abiotic stresses like drought. Field experiments performed on Oxisols in different Brazilian regions have shown that co-inoculation with *Bradyrhizobium* and *Azospirillum* improves the soybean grain yield in relation to the sole inoculation with *Bradyrhizobium* or supplemental mineral N (Hungria et al., 2015).

Lipo-chitooligosaccharides (LCOs) are microbial secondary metabolites (MSM) essential for communication and establishment of the *Bradyrhizobium*-legume symbiosis (Cullimore et al., 2001; Gough, 2003). Although not acting directly on the growth and development of the host plant, MSM stimulate the symbiosis and promote the microbial growth, among other effects (Davies, 1992). Under greenhouse conditions, the use of MSM of rhizobia increased the nodulation of soybean by 21% in number and by 12% in mass of nodules, whereas under field conditions, MSM associated with rhizobial inoculant increased the number of nodules by 23.6% and the grain yield by 4.8% as compared with the sole inoculation with *Bradyrhizobium* (Marks et al., 2013).

There are few studies on the combination of these sustainable technologies with the inoculation of *Bradyrhizobium* in soybean limited by drought. The aim of this study was to evaluate the effect of co-inoculation of *Bradyrhizobium* with *Azospirillum* and addition of LCOs on traits related to BNF and grain yield of soybean under different hydric regimes in field experiments.

2. Material and methods

2.1. Experimental sites

Four field experiments were conducted in 2013/14 and 2014/15 cropping seasons. In 2013/14, two trials were installed in Londrina, PR, Brazil in a clay soil (23°11′S, 51°11′W, 620 m a.s.l., Cfa Köpen-Geiger climate, Rhodic Eutrudox soil type, USDA), sown at different dates. The first, 14 Oct. 2013 is considered early sowing, while the second, 23 Nov. 2013 is considered late sowing in the regular local sowing calendar (EMBRAPA, 2010). In 2014/15, the trials were repeated in Londrina (sown on 4 Nov. 2014), and in a different edafoclimatic condition in Ponta Grossa in a sandy-loam soil (25°13′S, 50°01′W, 880 m a.s.l., Cfb Köpen-Geiger climate type, Typic Hapludox soil type, USDA) sown on 18 Nov. 2014. Both soils have previously established and naturalized populations of *Bradyrhizobium*, since they have been cropped with soybean for more than 30 years.

Before sowing, topsoil samples (0–20 cm) were collected for chemical, granulometric, and microbiological analyses (Table 1). The soil rhizobial populations were estimated by the most probable number (MPN) technique (Vincent, 1970), using soybean cultivar BRS 360 RR as trap plant.

2.2. Experimental design and treatments

The experimental design was in randomized blocks with six replications, and the following treatments: non-inoculated (Ni) control; Ni + N-fertilizer (100 kg ha^{-1} at sowing and 100 kg ha^{-1} at full flowering, as urea); Inoculated with *Bradyrhizobium* (I); Co-inoculated with *Bradyrhizobium* + *Azospirillum brasilense* (Co-I); Co-I + Microbial secondary metabolites (MSM); I + MSM. In 2013/14, the soybean cultivar was BMX Potência RR; in 2014/15, the cultivar was BRS 359 RR in both sites. Plots consisted of eight lines of 6.0 m in length spaced 0.50 m apart and density of 280,000 plants ha^{-1}.

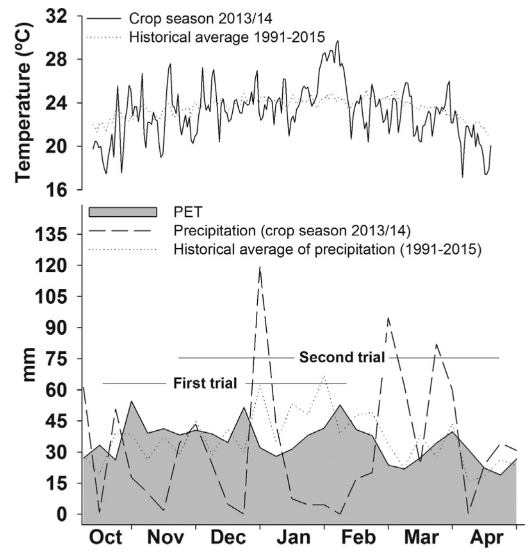

Fig. 1. Daily average temperature (°C), potential evapotranspiration (PET), precipitation (mm) every 7 days during the crop season period (2013/14), and historical (1991–2015) average temperature and precipitation in Londrina. Data on historical averages were adapted from Sibaldelli and Farias (2015).

The *Bradyrhizobium* inoculants contained the strains SEMIA 5079 (=CPAC 15) and SEMIA 5080 (=CPAC 7) of *B. japonicum* and *B. diazoefficiens*, respectively (with 1.2×10^9 colony forming units (CFU) mL^{-1}). The dose of inoculant was calculated to deliver 1.2×10^6 cells per seed. Treatments with co-inoculation received *Azospirillum brasilense* strains Ab-V5 and Ab-V6 (1×10^8 CFU mL^{-1}) in a dose to deliver 1.2×10^5 cells per seed. Treatments with MSM received the LCOs at 100 mL 50 kg^{-1} seeds, obtained as described in Marks et al. (2015).

2.3. Field management

Plants received 250 or 300 kg ha^{-1} of N–P–K (0–20–20) in furrow, simultaneously the sowing in both trials, in 2013/14 and 2014/15, respectively. No N-fertilizer was applied, except for the N-fertilized control plots. Seeds were not treated with fungicides or insecticides. At V4 stage (Fehr and Caviness, 1977), plants were leaf-sprayed with 20 g ha^{-1} of Mo ($Na_2MoO_4 \cdot 2H_2O$) and 2.5 g ha^{-1} of Co ($CoCl_2 \cdot 6H_2O$).

The potential evapotranspiration (PET), water balance for Londrina (calculated by Pan Evaporation Method, based on the average daily values for 2013/2014) and the mean daily temperatures and rainfall during the 2013/14 crop season trials, in addition to the historical average for rainfall and air temperature, are shown (Fig. 1). During the crop cycle in 2014/15, the mean temperature ranged between 19.1–29.0 °C and 15.1–27.5 °C, and the rainfall was 718 mm and 889 mm in Londrina and Ponta Grossa, respectively.

2.4. Plant sampling, analysis, and grain yield

The dynamics of nodulation were evaluated in the trials performed in 2013/14 crop season. Five plants were collected on the second and on the seventh line of the plot, i.e., 2 or 3 plants each side, within 0.5 m in the line, and the next sampling performed after 0.4 m forward to avoid effects of compensatory growth caused by gaps resulting from the previous sampling. Samples were taken at 10, 15, 25, 45, 65, and 95 days after emergence (DAE) for the first trial and at 12, 18, 27, 40, 70, and 115 DAE for the second trial, corresponding to V1, V2, V4, R2, R4-5, and R6-7 growth stages (Fehr and Caviness, 1977). The shoots were separated from roots, and dried at 65 °C for 72 h for assessment of shoot and root dry weights. The

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