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Review Paper

# A meta-analysis of the effectiveness of diverse rhizobia inoculants on soybean traits under field conditions

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ABSTRACT

Soybean is the most widely grown legume in the world. A beneficial feature of soybean is its ability to associate with rhizobia bacteria in its root nodules to fix atmospheric nitrogen through symbiotic nitrogen (N) fixation. Here, we provide a meta-analysis of 28 peer-reviewed studies examining the effectiveness of diverse rhizobia inoculants under field conditions, with the goal of understanding the underlying factors that affect inoculant success or failure. The data demonstrate that a diversity of *Bradyrhizobium* and *Sinorhizobium* species/isolates can be effective inoculants, including some indigenous strains. These inoculants varied in their efficacy for nodule number (−28 to +178 nodules), grain yield (−34% to +109%), and grain-N yield (−6% to +176%) compared to uninoculated controls. The greatest increase in nodule numbers occurred when background nodulation by indigenous soil rhizobia was absent or extremely low. Some studies demonstrated that indigenous rhizobia strains may be better adapted to local environmental stress conditions compared to introduced rhizobia, suggesting native rhizobia may have potential for local commercialization. There was a positive but moderate correlation between inoculant-mediated increases in nodulation and grain yield/grain-N. Moderate pH conditions were critical for inoculants to improve nodulation. Inoculant success was affected by the soybean genotype and soybean x rhizobia strain interactions, inoculant titre, formulation, and application method. Potential additional explanations from the literature for the failure/success of inoculants pertain to the persistence of rhizobia (inoculants) in soil, since seed-coated rhizobia colonize roots through the rhizosphere, and furthermore stress may favor indigenous, locally-adapted competitors. Rhizobia survival in soil is strain dependent and affected by soil organic matter, nutrients, pH, salinity, agricultural practices (e.g. organic, no till, rotations, application of pesticides) as well as temperature and drought. We conclude by proposing new studies to fill current research gaps.

## 1. Introduction

Soybean (*Glycine max* (L.) Merrill) originated in China and has been cultivated for more than 5000 years (Li et al., 2008). It is the top legume crop globally, representing 50% of the world's legume crop growing area (Herridge et al., 2008). Furthermore soybean is the top source of plant based protein (Nishinari et al., 2014). Major soybean producing countries in the world are the United States, Brazil, Argentina, China, and India (Biate et al., 2014; Chang et al., 2015). Soybean fixes atmospheric nitrogen through symbiotic nitrogen fixation (SNF), resulting in 16.4 Tg of nitrogen (N) fixed annually, accounting for 77% of the total N fixed by legume crops (Herridge et al., 2008). On average, SNF can meet 50–60% of the N demand of soybeans (Salvagiotti et al., 2008).

The process of SNF of soybean is complex, mediated by chemical communication between rhizobia and soybean to facilitate nodule formation and nitrogen fixation. Soybean plants produce isoflavones (genistein, daidzein) through the phenylpropanoid pathway which are released to the rhizosphere, where they trigger the nodulation (*nod*) genes in rhizobia (Subramanian et al., 2006). Once the *nod* genes are activated, rhizobia-to-plant signaling is mediated by the release of nodulation (*nod*) factors (D'Haeze and Holsters, 2002), causing root hair deformation wherein root hairs trap the rhizobia between the cell walls (Gage, 2004). Rhizobia enter into the plant roots with the initiation of an infection thread, which grows and directs bacteria towards the inner cortex of the roots (Jones et al., 2007). The infection thread releases bacteria into

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the cortical cells of the nodule primordium enveloped by a plant-derived membrane called the peribacteroid membrane (Mylona et al., 1995). Soybeans produce determinate type nodules, defined as nodules that lose meristematic activity after they initiate (Subramanian et al., 2007). Within each nodule, the released bacteria differentiate into bacteroids, which are the basic N fixing units enclosed by the peri-bacteriod membrane (PBM) and termed the symbiosome. Rhizobia multiply inside soybean root nodules, and some of the rhizobia can enter back into the soil once nodules senesce (Denison and Kiers, 2011).

For decades, it was thought that soybean could form nodules only in association with *Bradyrhizobium japonicum* (Rodríguez-Navarro et al., 2010). However, in time it was reported that soybean can also be nodulated by different species of *Bradyrhizobium*, as well as *Rhizobium*, *Mesorhizobium*, and *Sinorhizobium fredii* (Biate et al., 2014). Soybean nodulators include both slow growing rhizobia (*Bradyrhizobium* spp.) and fast growing rhizobia (e.g. *R. tropici*, *R. oryzae* and *M. tianshanense*) (Neves and Rumjanek, 1997; Hungria et al., 2001; Biate et al., 2014). According to de Almeida Ribeiro et al. (2015), currently there are 29 *Bradyrhizobium* species described in the literature: *B. japonicum*, *B. elkanii*, *B. liaoningense*, *B. yuanmingense*, *B. betae*, *B. canariense*, *B. denitrificans*, *B. pachyrhizi*, *B. jicamae*, *B. iriomotense*, *B. cytisi*, *B. lablabi*, *B. daqingense*, *B. huanghuaihaiense*, *B. oligotrophicum*, *B. rifense*, *B. arachidis*, *B. retamae*, *B. diazoefficiens*, *B. ganzhouense*, *B. paxllaeri*, *B. icense*, *B. manausense*, *B. ingae*, *B. valentinum*, *B. neotropicale*, *B. ottawaense*, *B. erythrophlei*, and *B. ferriligni*. The efficiency of SNF in soybean depends on the type of rhizobia strain that occupies the root nodules. Rhizobial strains vary in their nodulation ability, N fixation efficiency, compatibility with different crop cultivars, and adaptations to local environmental conditions (Biate et al., 2014).

Inoculation of soybean with rhizobia inoculants helps to improve soybean yield with low financial risk, as inoculants are cheaper than inorganic N fertilizers (Ronner et al., 2016). Soybean is increasing in importance in many developing countries, where poverty limits fertilizer use (Giller et al., 2011; Thilakarathna and Raizada, 2015). According to Giller (2001), there are three situations where introduction of rhizobia are necessary to ensure effective nodulation and SNF: (1) in the absence of compatible rhizobia; (2) when there is a low population of compatible rhizobia resulting in slow nodulation; and/or (3) ineffective or less effective indigenous rhizobia than the selected inoculants for a particular legume host variety. Inoculants may be especially required when soybean is introduced into a new geographic area, as compatible rhizobia may not be available in the soil. For example, when soybean was first introduced to North America (1765) and South America (1880–1882) (Chang et al., 2015), SNF was very low due to the lack of compatible and efficient rhizobia in local soils. However, with the introduction of promiscuous soybean varieties (those that nodulate freely with indigenous soil rhizobia), soybean may now be introduced into different environments even in the absence of suitable inoculants (Mpepereki et al., 2000).

Inoculant response is directly proportional to the available mineral N in the absence of local soil rhizobia (Thies et al., 1991a). However, inoculants also vary in their ability to compete against local rhizobia (Alves et al., 2003), as nodule occupancy varies between different rhizobia strains (Thies et al., 1992). Ineffective local rhizobia can compete with introduced rhizobia, leading to lower inoculant nodule occupancy (Sanz-Sáez et al., 2015). It was found that at least 66% of nodules must be occupied by a rhizobia inoculant in order to show a yield response in the presence of ineffective local rhizobia (Thies et al., 1991b). Furthermore, introduced rhizobia may not survive in the soil system due to competition from local, free living rhizobia, thus frequent inoculation may be required. However, the need for re-inoculation depends on the environment and particular rhizobia strain (Hungria and Vargas, 2000; Giller, 2001; Albareda et al., 2009b).

The native soil rhizobia population and its effectiveness for SNF can be highly variable between different soils (Singleton and Tavares, 1986; Thies et al., 1992, 1991b). Models based on analyses of field trial data suggest that the yield response is inversely related to the titre of the indigenous, compatible soil rhizobia population (Thies et al., 1991a). Therefore inoculation with effective rhizobia may not always improve SNF in some soil conditions, especially when the soil rhizobia population is above 20 cells per gram of soil and the population contains some effective strains (Singleton and Tavares, 1986). Thies et al. (1991b) have shown that rhizobia inoculants only show SNF or a positive yield response when the native rhizobia population is below 50 cells per gram of soil. The authors observed an 85% yield increase when the soil rhizobia population was below 10 cells per gram of soil.

The success of an introduced inoculant also depends on the quality of the inoculant (Rodríguez-Navarro et al., 2010; Ronner et al., 2016), wherein critical for successful nodulation are the number of viable rhizobia per unit of inoculant and the number of introduced rhizobia that result in root infection. Since the mobility of rhizobia in soil is limited under real field conditions, inoculation methods must ensure that sufficient rhizobia are present around the seeds for successful nodulation (Giller, 2001). However, the plant demand for N is determined by the yield potential of a crop in a given environment. If the N demand of soybean can be matched by the indigenous rhizobia population, inoculation with even efficient rhizobia strains may not show any improvement in yield or SNF (Thies et al., 1991a, 1991b). We have summarized the situations where inoculants are necessary and how legumes respond to inoculants under different soil conditions (Fig. 1) based on several key observations from the literature.

The survival and persistence of rhizobia are affected by soil and environmental factors (Hungria and Vargas, 2000). The survival of rhizobia in soil has been shown to be affected by extreme soil pH, desiccation, nutrient deficiencies, salinity/alkalinity, extreme temperatures, toxicities (Zahran, 1999; Hungria and Vargas, 2000; Giller, 2001), and predation by protozoa (Danso et al., 1975). Interestingly rhizobia can survive in the soil through formation of biofilms on biotic or abiotic surfaces (Hirsch, 2010; Denison and Kiers, 2011). The survival and titre of rhizobia in soil are positively correlated with soil clay content, percentage carbon, and moisture availability (Zengeni et al., 2006). Application of organic manure improves the survival of rhizobia in soil by improving the soil carbon and moisture content, thus minimizing the need for repeated inoculation (Zengeni et al., 2006). Screening and selection of rhizobia strains for different abiotic stresses under laboratory conditions may not work under field conditions, rather the laboratory findings provide possible explanations for the different SNF responses by rhizobia strains under abiotic stresses (Giller, 2001).

The genotypic variability among different rhizobia strains isolated from soybean growing soils and root nodules has been characterized primarily using molecular taxonomic analysis: Rep-PCR (Loureiro et al., 2007), RAPD characterization (Hungria et al., 2006), DNA microarrays (Itakura et al., 2009), amplified rDNA restriction analysis (ARDRA), multilocus sequence analysis (MLSA) (Li et al., 2011), BOX-PCR, Enterobacter repetitive intergenic consensus (ERIC) assays (Saldaña et al., 2003), 16S rDNA sequencing (Li et al., 2008), horizontal, fluorophore-enhanced, repetitive extragenic palindromic-PCR (HFERP) DNA fingerprinting (Wongphatcharachai et al., 2015), taxonomic analysis of various housekeeping genes (de Almeida Ribeiro et al., 2015), and sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis (Noel and Brill, 1980). Further sequencing of different symbiosis-requiring genes, including *nodA*,