Europ. J. Agronomy 72 (2016) 38–46

Contents lists available at ScienceDirect

European Journal of Agronomy

journal homepage: www.elsevier.com/locate/eja

Effects of soybean variety and *Bradyrhizobium* strains on yield, protein content and biological nitrogen fixation under cool growing conditions in Germany

Stéphanie Zimmer[a,d,*], Monika Messmer[b], Thorsten Haase[c,d], Hans-Peter Piepho[e], Anke Mindermann[d], Hannes Schulz[d], Antje Habekuß[f], Frank Ordon[f], Klaus-Peter Wilbois[g], Jürgen Heß[d]

[a] *Institute for Organic Agriculture Luxembourg (IBLA), 13 rue Gabriel Lippmann, 5365, Munsbach, Luxembourg*
[b] *Research Institute of Organic Agriculture (FiBL), Ackerstrasse 113, 5070, Frick, Switzerland*
[c] *Landesbetrieb Landwirtschaft Hessen, Kölnische Str. 48-50, 34117 Kassel, Germany*
[d] *Universität Kassel, Fachgebiet Ökologischer Land-und Pflanzenbau (FÖL), Nordbahnhofstr. 1a, 37213, Witzenhausen, Germany*
[e] *Universiät Hohenheim, Fachgebiet Biostatistik, Fruwirthstr. 23, 70593 Stuttgart, Germany*
[f] *Julius Kühn-Institut (JKI), Federal Research Centre for Cultivated Plants, Institute for Resistance Research and Stress Tolerance, Erwin-Baur-Straße 27, 06484, Quedlinburg, Germany*
[g] *Research Institute of Organic Agriculture (FiBL), Kasseler Strasse 1a, 60486 Frankfurt am Main, Germany*

ARTICLE INFO

Article history:
Received 7 April 2015
Received in revised form
15 September 2015
Accepted 20 September 2015
Available online 22 October 2015

Keywords:
Glycine max
Bradyrhizobium japonicum
Inoculation
Nodulation
Biological nitrogen fixation

ABSTRACT

Soybean (*Glycine max* (L.) Merr.) is able to fix atmospheric nitrogen in symbiosis with the bacteria *Bradyrhizobium japonicum*. Because these bacteria are not native in European soils, soybean seeds must be inoculated with *Bradyrhizobium* strains before sowing to fix nitrogen and meet their yield potential. In Central Europe soybean cultivation is still quite new and breeding of early maturing soybean varieties adapted to cool growing conditions has just started.

Under these low temperature conditions in Central Europe the inoculation with different, commercially available *Bradyrhizobium* inoculants has resulted in unsatisfactory nodulation. The aim of this study was: (i) to test the ability of commercially available inoculants to maximize soybean grain yield, protein content and protein yield, (ii) to study the interaction of different inoculants with different soybean varieties for two different sites in Germany under cool growing conditions over three years and (iii) to determine the variability of biological nitrogen fixation. Field trials were set up on an organically managed site at the Hessische Staatsdomäne Frankenhausen (DFH) and on a conventionally managed site in Quedlinburg (QLB) for three consecutive seasons from 2011 to 2013. Three early maturing soybean varieties—Merlin, Bohemians, Protina—were tested in combination with four different *Bradyrhizobium* inoculants—Radicin No.7, NPPL-Hi Stick, Force 48, Biodoz Rhizofilm—and compared with a non-inoculated control. Effective inoculation with *Bradyrhizobium* strains increased grain yield, protein content and protein yield by up to 57%, 26% and 99%, respectively. Grain yield, protein content and protein yield were generally higher in DFH. Average grain yield was 1634 kg ha^{-1} in QLB (2012–2013) and 2455 kg ha^{-1} in DFH (2011–2013), average protein content was 386 g kg^{-1} in QLB and 389 g kg^{-1} in DFH and average protein yield was 650 kg ha^{-1} in QLB and 965 kg ha^{-1} in DFH. The percentage of nitrogen derived from air (Ndfa) ranged between 40% and 57%. Soybeans inoculated with Radicin No. 7 failed to form nodules, and crop performance was identical to the non-inoculated control. Biodoz Rhizofilm, NPPL Hi-Stick and Force 48 are suitable for soybean cultivation under cool growing conditions in Germany. Interactions between soybean variety and inoculant were significant for protein content and protein yield at both sites, but not for nodulation, grain yield, thousand kernel weight and Ndfa. The variety Protina in combination with the inoculant Biodoz Rhizofilm can be recommended for tofu for both tested sites, while Merlin and Protina in combination with Biodoz Rhizofilm are recommended for animal fodder production in DFH. Animal fodder production was not profitable in QLB due to low protein yields.

* Corresponding author at: Institute for Organic Agriculture Luxembourg (IBLA), 13 rue Gabriel Lippmann, 5365, Munsbach, Luxembourg. Tel.: +352 26 15 13 84; fax: +352 26151386.
E-mail address: zimmer@ibla.lu (S. Zimmer).

http://dx.doi.org/10.1016/j.eja.2015.09.008
1161-0301/

1. Introduction

Soybean (*Glycine max* (L.) Merr.) is an important high-quality protein source for human and animal nutrition. With the soil bacteria *Bradyrhizobium japonicum* symbiotically colonizing the plant's roots, soybeans are able to fix atmospheric nitrogen (N_2). Because this bacterium is not native to European soils, soybean seeds are generally inoculated with *Bradyrhizobium* strains before sowing. Salvagiotti et al. (2008) reported in a review article that soybean biological N_2 fixation (BNF) ranged between 0 and 337 kg N ha^{-1} and on average 50-60% of soybean N demand originates from BNF.

Efficient symbiosis depends on environmental factors such as soil temperature, water and aeration, pH, salinity, amount of N in soil, as well as on the *Bradyrhizobium* strain, inoculation formulation, and soybean genotype (Keyser and Li, 1992; Stephens and Rask, 2000; Zhang et al., 1996). Thus, among other factors, nodulation is affected by low soil temperature and by inoculation procedure (Zhang and Smith, 1996; Zhang et al., 1996). A root temperature in the range of 25–30 °C is reported as optimal for BNF (Subramanian and Smith, 2013). In Central Europe soil temperature regimes is categorised as mesic, with mean annual soil temperature ranges between 8° and 15 °C (USDA-NRCS Soil Science Division, 2003). It is possible to select *Bradyrhizobium* strains and soybean genotypes to fix biological nitrogen efficiently for the environmental conditions in a given production area (Alves et al., 2003; Zhang et al., 2003). The combination of soybean variety and *Bradyrhizobium* strain can also be important, which was shown by Luna and Planchon (1995) and Solomon et al. (2012).

In Central Europe soybean cultivation is still quite new and breeding of early maturing soybean varieties adapted to cool growing conditions has just started. There are several different *Bradyrhizobium* inoculants available in Europe. However, they were mainly developed for the environmental conditions in the USA, and nodulation results after inoculation with these *Bradyrhizobium* inoculants have been unsatisfactory under low temperature conditions in Germany (Kadiata et al., 2012). Our hypothesis is that certain inoculants might be better suited for Central European growing conditions than others. To our knowledge, France is the only European country where the effectiveness of *Bradyrhizobium* inoculants has been tested before commercialization (Herridge et al., 2002), while no systematic field studies on the efficacy of commercial inoculation products with early maturing soybean varieties under cooler German farming conditions are available.

The aim of this study was: (i) to test the ability of commercially available inoculants to maximize soybean grain yield, protein content and protein yield, (ii) to study how different inoculants interact with different early maturing soybean varieties for two different sites in Germany under cool growing conditions over three years, and (iii) to determine BNF variability.

2. Material and methods

2.1. Site description

The experiments were conducted on two different sites in Germany: Hessische Staatsdomäne Frankenhausen (DFH) in Grebenstein, Hesse and Quedlinburg (QLB) in Saxony-Anhalt.

The field trials at DFH were operated under organic farming conditions. DFH is the research farm of the University of Kassel (51.4N; 9.4E) and is located 230 m above sea level. The farm was converted to organic farming in 1998 and is certified as organic by two organic farming associations, Bioland and Naturland. Soil type is a Haplic Luvisol and soil texture is a silty loam. Mean annual precipitation is 650 mm and the 30-year mean annual temperature is 8.5 °C. The average crop heat units calculated from sowing to harvest over the three experimental years 2011–2013 was 2953 °C (Brown and Bootsma, 1993).

The field experiments at QLB were operated under conventional farming conditions on the research station (51.4N; 11.8E) of the Julius Kühn-Institute. The research station is located 140 m above sea level and its soil type is a Chernozem with a loamy soil texture. Mean annual precipitation is 497 mm and the 30-year mean annual temperature is 8.9 °C. The average crop heat units calculated from sowing to harvest over the three experimental years 2011–2013 was 2525 °C (Brown and Bootsma, 1993).

2.2. Trial description

Field trials were conducted on both experimental sites in three consecutive seasons (2011–2013). Each year the soybeans were planted on different fields where soybeans had never been cultivated before. Three very early maturing soybean varieties (Merlin, maturity group (MG) 000; Bohemians, MG0000/000; Protina, MG000/00) were tested in combination with four different *Bradyrhizobium* inoculants (Radicin No. 7, NPPL-Hi Stick, Force 48, Biodoz Rhizofilm) and a non-inoculated control (Table 1). All three soybean varieties are cold tolerant varieties commonly cultivated under cool growing conditions in Central Europe. Merlin was chosen because this variety is the standard for stable grain yields under cool growing conditions. Bohemians variety is characterized as earlier maturing than Merlin and Protina is the earliest cold tolerant soybean suitable for tofu production.

A factorial treatment was arranged in a split-plot design with inoculant (I) as main plots and soybean variety (SV) as subplots. The main plot factor was laid out according to a randomized complete block design with four replications (REP). The subplot size was 15 m^2 (1.5 m × 10 m). Soybeans were sown with 65 kernels capable of germination per m^2 and a space between rows of 37.5 cm.

Seeds were inoculated according to the manufacturer recommendations (Table 1). For Radicin No. 7, concentration of bacteria per seed recommended by the producer was 100–400 folds lower than for the other inoculation products. Due to the unsuccessful inoculation in 2011, Radicin No. 7 was applied undiluted in 2012 (20 fold higher concentration than recommended). Inoculation was done just prior to sowing. For each plot the respective amount of inocula was added to the plastic bag containing the soybean seeds and thoroughly mixed. To avoid cross contamination, a thorough cleaning of the plot seeder was done after each inoculant, first by running 5 kg of barley seed though the plot seeder, followed by a thorough cleaning by air pressure.

Detailed descriptions of the trial management, soil characteristics, fertilizer and plant protection and time of sampling and harvesting are given in Table 2.

2.3. Measurements

To assess nodulation parameters, three (DFH) and four plants (QLB) per subplot were sampled from the second and the third row of each plot. Sampling was done twice, six weeks after sowing (nodulation1) and at flowering (nodulation2). The plants were carefully uprooted using a spade to obtain unharmed roots and nodules. The whole root system was exposed and the adhering soil was gently removed by hand over a metal sieve. The two subsamples from each subplot were used to assess nodulation (number of nodules per plant), record the inside color of nodules and to assess percent damage of nodules caused by pea and bean weevil (*Sitona lineatus*). There was no negative effect of *Sitona lineatus* damage, since *Sitona lineatus* was only observed for three plants in site DFH and year 2012.

At physiological maturity, soybean plants were harvested by a plot combine harvester. Grain yield measured in kilograms per