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Effect of previous cropping of rapeseed (*Brassica napus* L.) on soybean (*Glycine max*) root mycorrhization, nodulation, and plant growth

Lucio Valetti [a], Liliana Iriarte [b], Adriana Fabra [a, *]

[a] Departamento de Cs. Naturales UNRC, Ruta 36 km 601(5800), Rio Cuarto, Córdoba, Argentina
[b] INTA, Chacra Integrada experimental de Barrow, Ruta 3 Km 487 (7500), Buenos Aires, Argentina

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ABSTRACT

Arbuscular mycorrhizal fungi (AMF) improve the uptake of immobile mineral nutrients such as phosphate, thereby enhancing the growth of most plants. However, plants belonging to the Brassicaceae family such as rapeseed (*Brassica napus* L.) do not associate with AMF. In Argentina, one of the crops frequently used in rotation with rapeseed is soybean (*Glycine max* L.). The aim of this study was to evaluate the impact of the non-mycorrhizal rapeseed (inoculated with the phosphorus solubilizing bacteria *Bacillus* sp. LRCP-4 or *Arthrobacter* sp. LRCP-11) as the preceding crop, on soybean plants growth, nodulation and AMF colonization. Green house experiments were done using soil samples from rapeseed cultivated fields to growth soybean plants. Results indicated that the soybean interaction with the microsymbiont *Bradyrhizobium japonicum* E109 was not affected and that the growth of plants in soil previously planted with rapeseed inoculated with *Bacillus* sp. LRCP-4 was increased (31% and 29% for shoot and root fresh weight respectively). However, it was evident from this study that inclusion of rapeseed in the soybean-based system decrease by a 30% the AMF soybean root colonization.

1. Introduction

A very wide range of plant species can be colonized by soil fungi and form mutualistic symbiotic associations called arbuscular mycorrhizae (AM) with the exception of those that belong to the families Brassicaceae, Chenopodiaceae, and Caryophyllaceae [1]. Arbuscular mycorrhiza fungi (AMF) are members of the phylum Glomeromycota [2]. In this symbiosis the colonizing fungi benefit by accessing plant photosynthetic products and the plant by enhanced availability of soil water and nutrients [3]. Inoculation with AMF constitutes one of the major agronomic practices to improve this symbiosis in sustainable agriculture [4,5], and they are used as biofertilizers in a variety of crops [6]. AMF are obligate biotrophs and rely on their autotrophic host to complete their life cycle and to produce the next generation of spores [7]. Therefore, including non-mycorrhizal crops in rotation might affect the concentration and vitality of AMF species in soil, thereby affecting the growth of AMF-dependent crops following in the rotation [8].

Rapeseed (*Brassica napus* L.) belongs to the family Brassicaceae. It is an important oilseed crop worldwide, that ranks only behind soybean and palm oil in global production [9]. The oil found in rapeseed is used not only for food and fuel but also as raw material in the chemical industry. Furthermore, the residues from oil production are used as a valuable animal food providing a high energy and protein content [10]. Rapeseed seeds contain excellent edible oil that is lower in saturated fat and higher in omega-3 fatty acids than most other commercially available oils. These plants contain thioglucosides that produce isothiocyanates when tissues are disrupted, which affect negatively soil-borne fungal pathogens, and possibly AMF and other beneficial organisms, including rhizobial bacteria essential for legume root nodule formation and fixation of atmospheric di-nitrogen [11,12]. In Argentine, soybean crop is frequently used in rotation with rapeseed. The aim of this study was to evaluate the impact of previous cropping with rapeseed on soybean plants growth, nodulation and AMF root colonization.

2. Materials and methods

2.1. Growth and inoculation of soybean plants

Soybean seeds cultivar DM 4676 were superficially disinfected by immersion in ethanol (70%) for 1 min and in sodium hypochlorite (25%) for 5 min and washed six times with sterile distilled

* Corresponding author.
E-mail address: afabra@exa.unrc.edu.ar (A. Fabra).

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water [13]. Disinfected seeds were placed in Petri dishes with one layer of filter paper and moist cotton in an oven at 28 °C in the dark, until the radicle reached approximately 2 cm. Seedlings were sown in pots (360 cm^3, one seed per pot) containing a 1:1 mixture of vermiculite and soil collected from an agricultural field (located at Experimental Farm of Barrow, INTA, Argentina) where rapeseed seeds, inoculated with the phosphorus solubilizing bacterium *Arthrobacter* sp. LRCP-11 or *Bacillus* sp. LRCP-4, have been previously cultivated. For inoculation, 100 kg seeds were immersed and mixed with 800 ml of bacterial culture (1×10^9 cfu/ml). Uninoculated seeds were growing in phosphorus fertilized (diammonium phosphate 90 kg/ha) plots. Control soil samples were taken from unfertilized plots without previous rapeseed cultivation. After harvesting, soil from this agricultural field was randomly sampled at 25 points in each plot (depth, 0–20, 20–40 and 40–60 cm). All samples from each plot were combined. The experimental soil belongs to typic argiudoll. Physical and chemical soil properties were determined, at the beginning of the experiment, according to standard methods [14] (Table 1). The following treatments were evaluated: a) unfertilized soil samples from plots without previous rapeseed (T1), b) phosphorus fertilized soil samples from plots in which rapeseed plants developed from uninoculated seeds (T2), and c) soil samples from plots in which rapeseed plants developed from seeds inoculated with the phosphorus solubilizing *Arthrobacter* sp. LRCP-11 (T3) or *Bacillus* sp. LRCP-4 (T4) strains. Soybean seeds growing in pots containing the mixture of vermiculite and soil collected from the different plots at the agricultural field were inoculated with *Bradyrhizobium japonicum* E109 at planting time, according to Terouchi and Syono [15], by adding to each pot 1 ml of a bacterial culture obtained at exponential growth phase ($O.D._{620} = 1,1 \times 10^9$ cfu ml^{-1}) on Yeast Extract–Mannitol broth [16]. Plants were grown under controlled environment (light intensity of 200 μE m^{-2} s^{-1}, 16-h day/8-h night cycle, at a constant temperature of 28 °C and a relative humidity of 50%), and watered twice a week with Hoagland's nitrogen free medium [17]. At two months after planting, the following parameters were measured: (a) shoot and root fresh and dry weight; (b) number of nodules per plant; (c) nodule dry weight; (d) percentage of red nodules; and (e) root mycorrhizal colonization. All determinations were replicated with five separate samples.

2.2. Evaluation of soybean root colonization by AMF

Soybean roots were immersed in 10% KOH and heated at 90 °C for 1 h. Then, they were washed with water and immersed in a 1:1 solution of 10% KOH and 10% H_2O_2 for 5 min. After washing with water they were immersed in 1% HCl for 15 min. The HCl solution was discarded and the roots were immersed in trypan blue 0.05% at 90 °C for 15 min and washed. Finally, roots were immersed overnight in lactoglycerol solution (lactic acid: glycerol: water 1:1:1) [18]. Ten segments of about 1 cm length of each root were placed on a slide, and after the addition of two drops of lactoglycerol solution they were observed under microscope at 400X for estimation of AMF colonization [18]. Total AM fungal colonization measures the prevalence of all AM fungal hyphal structures in roots, indicating overall hyphal growth. Arbuscular and vesicles colonization specifically measures the prevalence in the root of these structures. All determinations were replicated with three separate samples.

2.3. Statistical analysis

Data were subjected to analysis of variance (ANOVA). Statistical significance was determined by Fisher's LSD (least significant difference) test at $p < 0.05$. Statistical analyses were performed using Infostat software version 2014e [19].

3. Results and discussion

Soybean biomass production was not affected by previous uninoculated rapeseed cultivation as no differences in root and shoot dry weights were determined between these plants and the control ones (Table 2). Moreover, it was interesting to find that previous cropping of rapeseed inoculated with *Bacillus* sp. LRCP-4 promotes soybean shoot and root fresh and dry weights, compared with plants growing in soil from uninoculated rapeseed seeded plots (Table 2).

The symbiotic association with *Bradyrhizobium japonicum* E109 seems not to be affected by the previous rapeseed cultivation since any differences was observed in the nodules number, nodules dry weight and percentage of red nodules between soybean plants growing in rapeseed cultivated or uncultivated soils samples. In a similar study, Muehlchen et al. [20] found a diminution in the peas (*Pisum sativum*) root nodule number when rapeseed shoot tissues were incorporated to soil before sowing. Other studies have also shown that root nodule formation and growth of rhizobial communities were affected by the incorporation of brassicas tissues to soil [21,22].

After rapeseed cultivation, irrespective of inoculation with

Table 2
Growth and nodulation parameters in soybean plants growing in soil from plots with or without previous inoculated or uninoculated rapeseed cultivation.

	Treatments			
	T1	T2	T3	T4
SFW (g)	2,81 ± 0,25 a	3,13 ± 0,15 ab	3,71 ± 0,18 bc	4,10 ± 0,26 c
SDW (g)	0,81 ± 0,08 a	0,86 ± 0,04 ab	0,92 ± 0.04 ab	1,01 ± 0,07 b
RFW(g)	1,38 ± 0,11 a	1,42 ± 0,13 a	1,60 ± 0,09 ab	1,84 ± 0,17 b
RDW (g)	0,28 ± 0,02 a	0,30 ± 0,01 a	0,30 ± 0,02 a	0,31 ± 0,02 a
NN	23 ± 1 a	21 ± 2 a	20 ± 2 a	22 ± 2 a
NDW (mg)	25,27 ± 3,43 a	31,48 ± 2,60 a	28,29 ± 2,16 a	27,70 ± 2,55 a
RN (%)	90,67 ± 5,36 a	91,25 ± 2,95 a	93,00 ± 3,55 a	94,44 ± 2,93 a

Soil samples from: plot without previous rapeseed, **(T1)**; plots in which uninoculated rapeseed was seeded, **(T2)**; plots in which *Arthrobacter* sp. LRCP-11 inoculated rapeseed was seeded, **(T3)**; plots in which *Bacillus* sp. LRCP-4 inoculated rapeseed was seeded, **(T4)**. **SFW**: shoot fresh weight; **SDW**: shoot dry weight; **RFW**: root fresh weight; **RDW**: root dry weight; **NN**: nodule number; **NDW**: nodule dry weight; **RN**: red nodules. The data are the mean ± SE of two independent replicates with five repetitions each. Means followed by the same letters are not significantly different ($P \leq 0.05$, Fisher's LSD).

Table 1
Soil physical and chemical properties.

Organic matter (%)[a]	NO_3–N (ppm)[b]	Humidity (%)[a]	Phosphorus (ppm)[a]	Sulfur (ppm)[a]	SO_4–S (ppm)[b]	pH[a]
2.9	0–20 cm: 59.6 ppm 20–40 cm: 40.2 ppm 40–60 cm: 16.6 ppm	5.0	32.2	312.6	0–20 cm: 17.1 ppm 20–40 cm: 13.5 ppm 40–60 cm: 12.9 ppm	5.7

[a] 0–20 cm depth.
[b] 0–20 cm, 20–40 cm and 40–60 cm depth.