



Appraisal of the crop-rotation effect of rhizobial inoculation on potato cropping systems in relation to soil bacterial communities

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ABSTRACT

Non-legume crops grown in rotation with legumes usually have reduced N-fertilizer requirement, which has both economic and environmental benefits. In this study, we aimed to assess the indirect effect of *Phaseolus vulgaris* inoculation with two indigenous rhizobia strains on potato growth promotion and disease control in relation to inoculation effects on soil bacterial communities. T-RFLP profiling demonstrated that inoculation significantly increased the phylotype richness of the bacterial communities at the end of *P. vulgaris* life cycle. A significant difference in richness between simple and dual inoculation was found. Effects on bacterial structure are clearly sensed with both inoculants. Various bacteria like *Halomonas*, *Arthrobacter*, *Rahnella*, *Actinobacterium* and *Frankia* were enhanced by inoculation irrespective of the inoculant type. However, other bacteria like *Clostridium*, *Bacillus*, *Stenotrophomonas* and *Xanthomonas* were enhanced by rhizobial mono-inoculation only and not by co-inoculation with both strains. Some bacteria may potentially behave like plant growth promoting rhizobacteria (PGPR) towards potatoes grown in rotation with common bean, as indicated by the 32% increase observed in potato yield, and also by the 56% decrease in potato wireworm infection. Therefore, rhizobia inoculation may contribute to the rotational benefits of legumes in potato cropping systems not only by providing fixed nitrogen, but also by increasing microbial diversity and structure, potentially stimulating plant growth promoting rhizobacteria and enhancing disease control. However, these effects depend largely on inoculant formulation.

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1. Introduction

Explaining factors that drive the diversity, distribution and abundance of species is one of the central themes in ecology. While understanding the diversity, distribution and abundance of large organisms is already a difficult task (Gaston, 2000). This task is considerably more complex for microbial ecologists, considering the small size and staggering diversity and abundance of microorganisms (Torsvik et al., 1990; Curtis et al., 2002; Gans et al., 2005). Several factors have been put forth to explain patterns of soil microbial community composition, including spatial variation, soil type, nematodes and vegetation (Wardle et al., 2004; Martiny et al., 2006). In vegetated areas, it is supposed that soil resources for microbial growth are mainly controlled by plant inputs through the deposition of litter and root exudates (Wardle et al., 2004).

However, factors other than plants have been also reported as drivers of microbial community composition. For instance, abiotic and biotic soil factors, such as pH, salinity and biofertilizers, were reported as being the most important in influencing microbial community composition (Fierer and Jackson, 2006; Lozupone and Knight, 2007; Trabelsi et al., 2009, 2011).

Symbiotic N₂-fixation by legumes is generally believed to be the dominant source of biological nitrogen input in the biosphere. Nitrogen chemicals account for as much as 30% of the total fertilizers needed for agricultural crops (Tran et al., 2006). With the increasing cost of chemical fertilizers and concern about environmental pollution, the role of biological nitrogen fixation in sustainable agriculture, has to be elucidated more in deep. The benefits of inoculation may also be mediated to the non-legume crops grown in rotation by increasing yields and reducing disease infestation, probably through stimulating microbial diversity and activities (Sturz and Kimpinski, 2004).

Potato is one of the most important crops in the world. Like for most agricultural crops, large amounts of nitrogen inputs are

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usually used in potato cropping systems. The yield is also compromised by numerous disease attacks, including entomopathogenic nematodes, which can result in major economic losses. The most destructive of soil insect pests of potatoes and other crops are wireworms (larvae or juvenile form of the click beetles, *Coleoptera*, Elateridae). Their importance as crop pests seems to be increasing; however, chemical control was found to be ineffective (Parker and Howard, 2001). Crop rotation is believed to help in overcoming some of these problems. However, little is known about the indirect effect (as PGPR) of legume inoculation on potato cropping systems, which requires further clarification. In a previous work (Trabelsi et al., 2011), we assessed the effect of inoculation of common bean with two rhizobial strains on soil bacterial communities. T-RFLP profiling demonstrated that inoculation significantly increased the phylotype richness. Effects on bacterial structure and diversity were clearly sensed in the bulk soil. The aim of this work was then to evaluate more in deep the extent of these effects on potatoes grown on the same plot, particularly the outcome on growth promotion and disease control of wireworms.

2. Materials and methods

2.1. Experimental design

The on-field experiment was carried out in an experimental plot of the Technical Centre of Potatoes located in the region of Manouba, North Tunisia (36° 48' 28" North, 10° 6' 4" East). The soil was non-saline and slightly alkaline and had been used for potatoes, cereals and faba bean cultivation but had not been cropped since one year. The soil is a fluvisol with 55% clay, 20% silt, 25% sand, 2.5% humus, 1.2 g/Kg total nitrogen, and pH 8.5. The two indigenous rhizobial strains (*Rhizobium gallicum* 8a3, *Ensifer meliloti* 4H41) used for inoculation were previously isolated from common bean nodules from Tunisian soils (Mrabet et al., 2005; Mnasri et al., 2007a). Inoculation details and design of the common bean crop-rotation-step of this experiment have been described by Trabelsi et al. (2011). The experimental design consisted of five treatments, not-inoculated, nitrogen-fertilized, and inoculated with *E. meliloti* 4H41, *R. gallicum* 8a3 or with both strains (½, ½). The experiment was conducted in a completely randomized block design. Each treatment was subdivided into three randomized subplots (5 m/6 m in size) cultivated with *Phaseolus vulgaris* cv. Flamingo in the first campaign (March to June 2009), followed by potatoes on September–December 2009. During the first campaign (common bean), the nitrogen-fertilized control received the equivalent of 100 kg of KNO₃/ha fractioned at two times, two-leaf seedlings stage and beginning of pod formation. All treatments received the equivalent of 4 kg/ha of chelated iron by foliage spraying. At the end of this culture (June), soil samples were collected for T-RFLP analysis. By September, land preparation was carried out by tractor ploughing followed by harrowing. Each subplot was tilled separately in order to avoid treatment mixing. The cultivar Spunta of potato was then sown at a density of 4.16 plants/m². All subplots (5 treatments replicated thrice) received the equivalent of 225 kg of ammonium nitrate/ha (50% of the recommended dose), 150 kg ha⁻¹ of P₂O₅ and 360 kg ha⁻¹ of potassium sulphate. The experiment was carried out under drip irrigation and weeds were removed manually.

2.2. Growth parameters and wireworm infestation

At flowering stage, 30 plants (10 from each replicated subplot) were randomly collected from each treatment and used for nodule counting. At final common bean harvest, 90 plants were collected from each treatment (30 from each replicated subplot) and used for

grain yield determination. At final potato harvest, 30 plants were collected from each treatment (10 from each replicated subplot) and used for potato yield determination. The wireworm infection was evaluated as the percentage of the potato infected yield of 300 plants from each treatment (100 plants from each replicated subplot). Statistical analysis was performed using the STATISTICA software by the ANOVA/MANOVA program. The HSD Tukey's test ($p < 0.05$) was used for mean comparison.

2.3. Soil sampling and DNA extraction

At the end of the first crop-rotation step, root-free soil samples (15 cm of topsoil) were taken with a soil corer at the middle of the rows (25 cm from each) in five replicates and consciously checked to ascertain the absence of roots. The samples were then combined and homogenized and stored at –80 °C for later DNA extraction after shock-freezing in liquid nitrogen. DNAs from each of the 15 samples (5 treatments × 3 subplots) were extracted as previously reported (Trabelsi et al., 2011) and stored at –20 °C until use.

2.4. T-RFLP analysis of the total-bacterial soil communities

The bacterial 16S rRNA genes were amplified using the universal bacteria specific primers 27f and 1492r (Lane, 1991). The forward universal primer 27f was labelled with HEX (hexachloro-carboxy-fluorescein). T-RFLP was performed with *Rsa*I and *Msp*I restriction enzymes as previously reported (Trabelsi et al., 2011). An ABI 310 capillary DNA sequencer was used to analyze the labelled DNA fragments. The fragment size was determined on the basis of an internal size marker (MapMarker ROX (35–500 bp) using ABI Genescan[®] software. Analysis of T-RFLP profiles and putative taxonomic assignment of Terminal-Restriction Fragments (TRFs) was performed as previously described (Trabelsi et al., 2011). TRFs outside the marker size range were not considered for analysis. Derivative T-RFLP profiles of the different enzymes were combined together and transformed to a binary vector (1, 0), in which presence or absence of each TRF in any of the three replicated subplots of each treatment (1/3, 2/3 or 3/3) was scored. The vectors of binary profiles were then compared to compute the similarity matrix using the Jaccard index and then used to construct an UPGMA dendrogram using the Past 2.02 software package (Hammer et al., 2001).

3. Results

3.1. Effect of crop rotation on potato yield

Inoculation of common bean induced a significant increase in nodule number and grain yield with both strains. However, *R. gallicum* strain 8a3 showed the highest nodule number and grain yield. Co-inoculation with a mixture of both strains (½, ½) gave the same yield on common bean as the simple inoculation with strain 4H41. A significant crop rotation effect was also observed on potato yield with both strains even if all subplots received the same 50% of the recommended nitrogen input (Table 1). Nevertheless, the potato yield significantly decreased when both strains were co-inoculated as a mixture. The non-inoculated subplots showed the lowest yield.

3.2. Effect of crop rotation on potato wireworm attacks

The potato infestation by wireworms was evaluated as the percentage of potato infected yield (Table 1). The inoculation crop-rotation-effect induced a significant decrease of the wireworm attacks comparing to the non-inoculated and the nitrogen-

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