Effects of arbuscular mycorrhizal fungus *R. irregulare* and the bacteria *B. amyloliquefaciens* on plant growth

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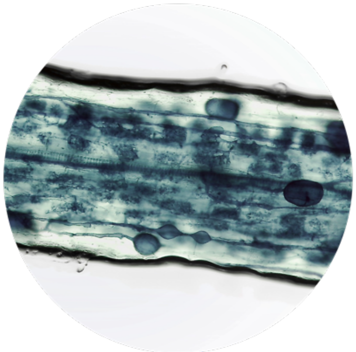

Native arbuscular mycorrhizal fungi colonizing the roots of root parsley.

ABSTRACT

Due to high standards for product quality of vegetables, soils under vegetable production are managed extremely intensively, and are characterized by high inputs of fertilizers, as well as high frequency in tillage, milling and hoeing. In consequence vegetable production can have seriously negative impacts on soil health and ecosystem services. Through their fungal network arbuscular mycorrhizal fungi (AMF) can provide nutrients for plants and therefore represent a promising solution to reduce fertilizer inputs.

In this study a field trial was carried out with the crop root parsley on a farm with organic management. The effect of the two biostimulants *Rhizoglomus irregulare* and *Bacillus amyloliquefaciens* inoculated alone or in combination, on yield performance was evaluated by measuring the plant weight, colonization rate by the inoculated fungus in plant roots, and plant nutrient concentrations.

A positive effect of the inoculation with *R. irregulare* on plant growth was found, with a significant yield increase of 31 %. Both inoculated and control plants showed high root colonization levels, indicating that the occurrence of native AMF in the soil was already high. No significant positive effects of *B. amyloliquefaciens* on plant growth were found. The yield increase by *R. irregulare* inoculation in root parsley was not reflected in a higher colonization rate by AMF in the roots. However, analysis of the nutrient concentrations of the experimental plants showed, that inoculated plants presented significantly higher uptakes of N, P, Mg and Ca. Therefore it is assumed that the inoculated AM-fungus has replaced the native AM-fungi in the root.

The results imply that in the future molecular methods present promising tools to investigate the arbuscular mycorrhizal root colonization at species level and therefore, to better understand the influence of inoculated AM- fungi.

1 Introduction

1.1 AM fungi and bacteria in agricultural systems

Arbuscular mycorrhizal fungi (AMF) form a widespread symbiotic interaction with plants leading to benefits for the plant in terms of growth, nutrition and protection (Bender et al. 2019). Through intimate associations, AMF can provide up to 80% of the phosphorus needs of the host plant (Smith and Read 2010). It is likely that the long co-evolution of plants and AMF did not occur independently from the associated bacterial flora (Frey-Klett et al. 2007). In some plant species, AMF colonization may even strongly depend on the presence of mycorrhiza helper bacteria (Xie et al. 2018). Co-inoculation of *B. amyloliquefaciens* and AMF have resulted in positive effects on growth and yield parameters (Yusran et al. 2009; Mikiciuk et al. 2019). These results suggest that selected bacteria in the rhizosphere of plants and AMF could be co-inoculated to optimize the formation and functioning of the AMF symbiosis.

1.2 Study overview

The objective of this study was to evaluate the influence of AMF (*Rhizoglomus irregulare* strain SAF22) inoculation, alone or in combination with *B. amyloliquefaciens*, on plant growth of root parsley *(Petroselinum crispum subsp. Tuberosum).* In this regard a field trial on a farm with organic management in the north-east of Switzerland was carried out. The results of this study will help to develop sustainable farming methods in vegetable production with the aim to reduce the use of fertilizers.

It was hypothesized that improved root colonization by single or combined application of *R. irregulare* strain SAF22 and *Bacillus amyloliquefaciens* strain FZB42 will enhance plant growth of root parsley. Specifically, the present study poses the question whether single and/or combined treatments with *R. irregulare* and *B. amyloliquefaciens* produce higher yields compared to control plants.

2 Material and Methods

2.1 Biostimulants and experimental design

The two biostimulants AMF *R. irregulare* strain SAF22 from the Agroscope collection and *B. amyloliquefaciens* (product RhizoVital 24 from Andermatt Biocontrol) were inoculated alone or in combination. In total, the four treatments AMF inoculum (M), AMF control inoculum (MC) Bacillus and AMF inoculum (BM) and Bacillus with AMF control inoculum (BMC) were applied.

For the experimental design, each of the four treatments was assigned a plot of 3 meters in length. Each treatment was replicated six times, arranged in a completely randomized block design, with a total of 24 plots.

2.2 Mycorrhizal growth and nutrient uptake response

Mycorrhizal growth response (MGR) was calculated as described in (Köhl et al. 2016) as a measure for the percentage change in plant biomass in plots inoculated with the AM fungus, relative to mean biomass of control plots. Likewise, mycorrhizal nutrient uptake (MUR) of the plants was calculated.

2.3 Statistical Analysis

Data was managed using Microsoft Excel 2020 for Mac. For statistical analysis, the software R Studio version 1.3.959 was used (R Core Team 2020). The significance level for all tests was set at alpha = 5%. Normal distribution was checked using QQ-Plots (visual analysis), as well as Kolmogorov-Smirnoff and Shapiro-Wilk tests. Comparisons of the different treatments were performed using two-way ANOVAs if the normal distribution of the residuals was given.

The package multcomp was used for multiple comparisons analysis (Hothorn et al. 2008). In case of significant ANOVA results post hoc analysis was performed using pairwise Tukey tests. Results of post hoc analysis were translated into letters. Different letters show significant mean differences ($p < 0.05$) according to Tukey test.

The package lme4 was used to perform a linear mixed effects analysis of the relationship between the different parameters and treatment (Bates et al. 2015). As fixed effects, two factors AMF and Bacillus (with interaction term) were entered into the model. As random effects, the intercept for block was given.

The package sciplot was used for data visualizations (Morales and Murdoch 2020). Results were visualized using bargraphs. Correlations between biomass and root colonization were performed using the Pearson correlation coefficient, if data was normally distributed. For not normally distributed variables the Spearman rank correlation coefficient was used.

Significant differences of the mycorrhizal growth response (MGR) and mycorrhizal uptake response (MUR) from control mean values were assessed by one sided t-tests.

3 Results

In this chapter, the influence of *R. irregulare* and *B. amyloliquefaciens* applied alone or in combination on plant growth of root parsley is shown. Furthermore, the establishment of *R. irregulare* in the root is shown, and how root colonization rates differ between treatments. Effects on nutrient uptake are presented with the mycorrhizal nutrient uptake response.

3.1 Effect on plant yield

In root parsley, the total dry and fresh biomass of the harvested plants within 1 meter was evaluated. With a yield increase of 31 %, root dry weight was significantly higher ($p = 0.03$) in the treatment with *R. irregulare* (M) compared to the control treatment (MC) (Fig. 1).

We also calculated the mycorrhizal growth response (MGR), to visualize the percentage change in root fresh and dry biomass, compared to control treatments. In root parsley a significant growth response of inoculated plants with a 23% change in root fresh biomass was found, compared to the mean of control plants (Fig. 2). Both fresh and dry root biomass differed significantly from the mean of control treatments ($p = 0.007$, $p = 0.001$).

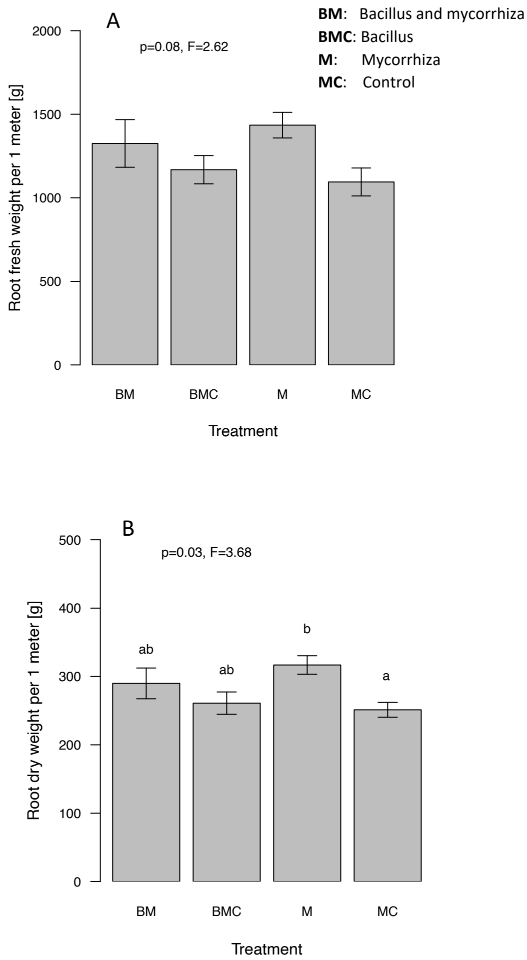

Figure 1: Effects of inoculation by the treatments BM, BMC, M and control (MC) in root parsley on (A) produce fresh and (B) produce dry weight. ANOVA results for the effects of inoculation on fresh and dry produce weight are given. In (A) a tendency of higher yield of (M) in comparison with the control (MC) can be observed ($p = 0.080$, $F = 2.62$). In (B) a significant increase in dry weight with treatment M was found ($p=0.030$, $F=3.68$). Different letters show significant mean differences ($p<0.05$) according to Tukey test. Bars represent means of 6 replicated plots per treatment +/- 1 SE.

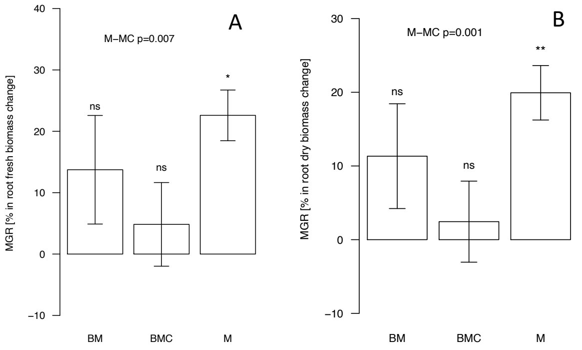

Figure 2: MGR of treatments M, BM and BMC in (A) fresh and (B) dry root weight of root parsley (p=0.007, p= 0.001). Significant differences from the mean value of control treatment were assessed by one-sided t-tests. Test results are given above bars as ns > 0.1, * p < 0.05 and ** p < 0.001. Bars represent means of 6 replicated plots per treatment (five replicated plots for treatment BM) +/- 1 SE.

3.2 *Effect on root colonization by AMF*

Establishment success of the inoculated fungus was assessed by comparing root colonization parameters including (A) total, (B) vesicular and (C) arbuscular colonization (Fig. 3). A tendency towards higher total and vesicular root colonization in plants treated with *R. irregulare* is visible, but no significant effects were observed. Average total root length colonized in control plots (MC) shows a mean total root colonization level of 58%.

3.3 *Effect on nutrient uptake*

In the field trial with root parsley, a significantly higher uptake of the nutrients N, P, Mg and Ca was observed in plants treated with *R. irregulare* compared to the control (Fig. 4). The calculation of the mycorrhizal uptake response (MUR) of the nutrients showed an increase in the concentration of 20 % in P and Mg , 19% in N, and 17% in Ca. For the nutrients potassium and sodium, no significant nutrient uptake response was observed (results not shown).

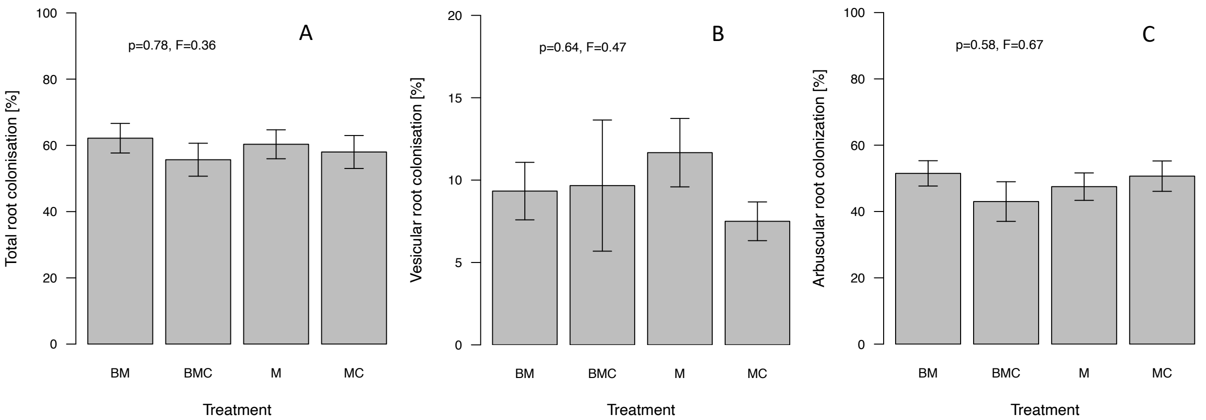

Figure 3: In root parsley no significant effects of treatments M, BM and BMC on (A) total, (B) vesicular and (C) arbuscular root colonization levels were observed. Bars represent means of 6 replicated plots per treatment +/- 1 SE.

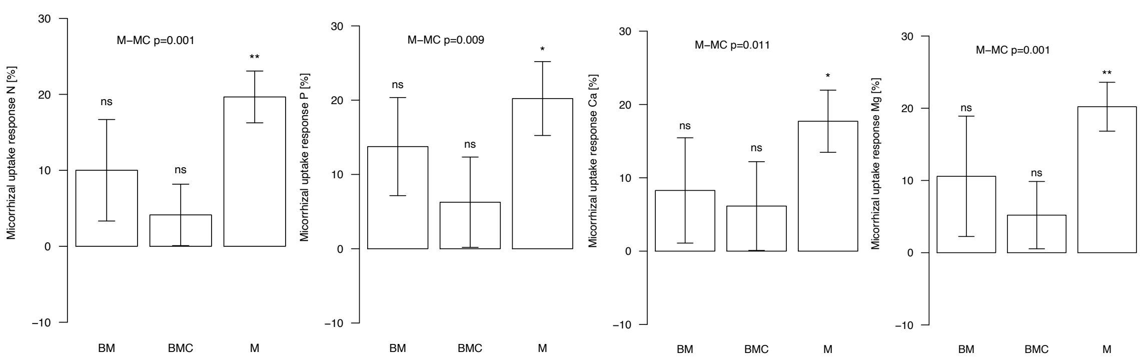

Figure 4: Mycorrhizal uptake response showing the significant percentage change of nutrient concentrations of N, P, Ca und Mg in root parsley roots inoculated with the treatments BM, BMC and M. Differences from the mean value of control treatments were assessed by one-sided t-tests. Test results are given above bars as ns > 0.1, * p < 0.05 and ** p < 0.001. Bars represent means of 6 replicated plots per treatment (five replicated plots for treatment BM) +/- 1 SE.

4 Discussion

The aim of this work was to find out whether the well-characterized and efficiently colonizing AM fungus *R. irregulare* can establish in the roots of root parsley and can increase crop yield under field conditions. In addition the study investigated if the combination with *B. amyloliquefaciens* has additional positive effects on plant growth. In the following the first chapter discusses the the effect of single and combined inoculation of the two biostimulants on plant growth and nutrient uptake. The second chapter is devoted to the evaluation of the establishment of the inoculated fungus. This is followed by a reflection of limitations of the study. An outlook on the implications in practice concludes the work.

4.1 Effect on plant growth and nutrient uptake

A significant increase of biomass of 31% in root parsley was detected. The results of the mycorrhizal growth response confirmed this increase, with a MGR of 23% in root parsley. The influence of *R. irregulare* was also demonstrated in the nutrient uptake with a significant mycorrhizal uptake response of the nutrients N, P, Mg, and Ca in AMF inoculated plants. In addition a tendency to a positive influence of the combined inoculation with Bacillus and AMF compared to the control was observed. However, the yields of the combined inoculation did not significantly exceeded the yields of the single inoculation with *R. irregulare*. Therefore it remains to be validated whether the colonization by *R. irregulare* is particularly facilitated, if applied in combination with the bacteria *B. amyloliquefaciens*.

4.2 Establishment of the inoculated fungus

The examination of root colonization demonstrated a symbiosis of root parsley with AM fungi. High colonization levels by AMF were detected in both inoculated and control plants, with 59% of root length colonized by native AM fungi in control plants. It is assumed that the investigated field already reached the field carrying capacity for AMF and therefore the inoculation with *R. irregulare* showed no significant increase in root colonization levels. These results confirm the findings of earlier studies, concluding that the establishment of inoculated AMF is dependent on abundance of native AMF in the soil. A lower abundance of native AMF communities in the soil may favor the establishment of inoculated AMF because more niche space is available for inoculated AMF (Niwa et al. 2018; Bender et al. 2019).

Despite the positive effect of *R. irregulare* inoculation on biomass in root parsley, no significant increase in root colonization compared to control could be detected in this experiment. Therefore, the effect on biomass can not be confirmed with the results of root colonization. Bender et al. (2019) however, demonstrated that the quantitative real time polymerase reaction (qPCR) method can detect an increase of the inoculated AMF in the plant root without this being reflected in an increase in total root colonization levels. This could be based on the fact that inoculated AMF replace native AMF in the root (Schlaeppi et al. 2016). However, this assumption raises concerns about the inoculation of AMF as they may behave invasively and thus interfere with native AMF communities.

4.3 Limitations of the study

To achieve more statistical power, it would be interesting to repeat this type of field trial with a larger number of replicates and at multiple sites, including fields with demeter and conventional management. Since no significant results were obtained in this trial with *B. amyloliquefaciens* as MHB for AMF, the co-inoculation of Bacillus, as well as other MHB with AMF under field conditions, should be further investigated.

A limitation of this bachelor thesis was the detection methods of the inoculated biostimulants. On the one hand, the colonization by AMF in the root was not assessed with molecular methods on a species level. This made it difficult to draw reliable conclusions about the establishment success of the inoculated AM fungus. On the other hand, no investigations were carried out on the colonization of the root by *B. amyloliquefaciens*. Therefore, the influence of *B. amyloliquefaciens* could only be determined by plant weight.

Furthermore, the investigation of native microbial communities in the soil and their influence on the inoculated fungus and Bacteria represents an exiting area for future research.

5 Outlook and implementation in praxis

Promoting AMF in the soil could be profitable for vegetable farms. The trial results show that AMF has the potential to increase yields and that in the future molecular methods present promising tools to investigate the arbuscular mycorrhizal root colonization at species level.

In severely degraded soils, inoculation may provide a solution strategy to reestablish AMF in the soil (Asmelash et al. 2016). Similarly, inoculation of AMF can be integrated into crop rotations by adding them after crops that do not form symbiotic relationships with AMF (e.g., crucifers) (Berset et al. 2011).

To promote native AMF preferably, soils are inoculated with native mycorrhizal fungi from the farms own field (Pellegrino et al. 2011). Douds et al. (2010) have developed a method for propagating native AMF on the own farm. In the future, it will be important to develop further strategies to facilitate AMF application in the field.

The importance of the promotion of native AMF should not be neglected when considering to use the benefits of these fungi. For example, constant root penetration and low tillage intensity of the soil, avoidance of pesticides and the most diverse crop rotation possible are viable strategies for enhancing root colonization by native AMF (Mäder et al. 2000; Berset et al. 2011; Köhl et al. 2014; Bowles et al. 2017; Helander et al. 2018). Through such measures, internal regulatory ecosystem functions can be promoted and AM fungi can thrive in the soil for longer periods of time and thus provide the desired benefits (Zhang et al. 2020).

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