THE EVALUATION OF RHIZOBIUM LEGUMINOSARUM STRAINS EFFECTIVENESS IN FIELD BEANS (VICIA FABA L.) AT DIFFERENT SOIL MICROBIOLOGICAL ACTIVITY

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Abstract
The inoculation of the legume seed material with active nitrogen fixing bacteria strains before sowing has a significant role for the increase of the legume yield. Inoculation can improve crop yields in cases where appropriate rhizobia are not present in the soil or the soil contains a significant proportion of non-nodulating or ineffective nitrogen-fixing strains. The aim of the investigation was to detect the effectiveness of Rhizobium leguminosarum strains in field beans at different soil microbiological activity. The experiment was conducted at the Institute of Soil and Plant Sciences of the Faculty of Agriculture of the Latvian University of Agriculture from the 5th of June till the 17th of October 2008.

The field bean (Vicia faba) cultivars ‘Ada’, ‘Lielplatones’, Rhizobium leguminosarum bv. vicia strains No. 110; 408; 501 and 2 types of soils (with different microbiological activity) were used in vegetation pot experiment. The highest shoot dry matter was observed in cultivars cultivated in soil with higher microbiological activity. The highest pod dry matter was observed in cultivars cultivated in soil with higher microbiological activity. The highest total nitrogen amount was in field beans cultivated in soil with lower microbiological activity. Rh. leguminosarum strain resistance to streptomycin decreases with the plants age, from anthesis forward in both soil types and both cultivars. The fingerprinting showed significant difference between Rh. leguminosarum strains.

Key words: field beans, Rhizobium leguminosarum, inoculation.

Introduction
Wide cultivation and spread of field beans (Vicia faba L.) in the temperate and the subtropical regions have ranked it the fourth most important legume crop in the world, next to dry beans, dry peas and soya. The crop contributes to human and productive domestic animal nutrition as a result of its high protein content and other essential nutrients. Although field beans are less consumed as human food in western countries, it is considered as one of the main sources of cheap protein and energy in Africa, parts of Asia and Latin America, were most people cannot afford meat sources of protein (Haciseferogullari et al., 2003).

The nutritional value of field beans has always been traditionally attributed to its high protein content, which ranges from 27 – 34% depending on genotypes. Most of these proteins comprise globulins (79%), albumins (7%), and glutelins (6%) (Haciseferogullari et al., 2003). Legume seeds contain several comparatively minor proteins, including trypsin inhibitors, lectins, lipoxgenase and urease, which are relevant to the nutritional quality of the seed (Alghamdi, 2009).

One of the major aims of agricultural policy is increasing crop production, which could be achieved by various ways; one of the methods is increasing the efficiency of biological nitrogen fixation.

Rhizobium is a genus of soil bacteria whose members are best known for their ability to establish symbiotic relationships with legumes of agricultural and environmental importance in a process of biological nitrogen fixation (Moschetti et al., 2005).

Symbiotic nitrogen fixation results from the complex interaction between the host plant and the microorganisms. The host plant provides the microorganisms with a source of energy for growth and function, and with a specialized ecological niche. The microorganism fixes atmospheric N₂ and provides the plant with a source of reduced nitrogen, in the form of NH₃ (Hirsch, 1992).

The inoculation of cultivated leguminous plants with selected rhizobial strains is recommended in order to maximize the contribution of biological nitrogen fixation to the nitrogen status of the host plant (Oliveira et al., 1999).

The success of inoculants requires that the inoculant strains are both highly effective in nitrogen fixation and highly competitive with the indigenous soil strains in nodule formation (Gwyn et al., 1989). The presence of indigenous rhizobia in soil may represent a barrier to efficient inoculation with Rhizobium leguminosarum strains because indigenous strains are often better adapted to the prevailing soil and climate condition.
(Oliveira et al., 1999). The various environmental and biotic factors which directly or indirectly affect competition for nodulation of the host legumes, including soil characteristics, pH, salinity, water potential, temperature, microbial antagonism, predation and parasitism (Bottomley, 1992).

The aim of the investigation was to detect the effectiveness of *Rhizobium leguminosarum* strains in field beans in soils with different microbiological activity.

### Materials and Methods

The experiment was conducted at the Institute of Soil and Plant Sciences of the Faculty of Agriculture of the Latvian University of Agriculture from the 5th of June till the 17th of October 2008.

The field bean (*Vicia faba. L.*) cultivars ‘Ada’ and ‘Lielplatones’ were used in vegetation pot experiment.

Before seeding, the surface of field bean seeds was sterilized (with ethanol, 98%), rinsed three times in sterile water. *Rhizobium* seed inoculation was done by using *Rhizobium leguminosarum* bv. *vicia* strains No. 110; 408; 501 which were obtained from the *Rhizobium* collection of the Latvia University of Agriculture Institute of Soil and Plant Science. The *Rh. leguminosarum* strains No. 110 are included in IBP World catalogue of *Rhizobium* leguminosarum Collections (Allen and Hamatova, 1973). The inoculants were mixed with moistened seeds. Control seeds weren’t inoculated with *Rh. leguminosarum* strains.

The vegetation pots (5 L) were sterilized (with phenol, 5%) and after sterilization filled with soils. In each pot were seeded five sterile field bean seeds. In the vegetation pot experiment were used 2 types of soils from the Latvia University of Agriculture agency research institute of soil and plant sciences. The first type (a): organic matter 2.7%; pH KCl 6.8; P 60.66 mg kg⁻¹; K 86.33 mg kg⁻¹ and the second type (b): organic matter 5.2%; pH KCl 6.9; P 71.13 mg kg⁻¹; K 82.18 mg kg⁻¹. The soil microbiological activity was detected by catalysis. Results show: the soil A catalysis activity on legumes root infection and competitiveness (Bottomley, 1992).

### Results and Discussion

The obtained results on resistance to the antibiotic streptomycin was analysed at butonisation, anthesis and pod development stages. At least 20 nodules from each inoculated sampling at each plant development stage and a total of 3200 nodules were processed for strain identification.

Nodules were washed and surface sterilized by sublimate (HgCl₂ concentration 0.1%) for 5 minutes. The nodules were then washed in three changes of sterile distilled water, crushed and streaked on maximum sustainable yield agar containing streptomycin (100 µg mL⁻¹) and incubated at 27 °C. For the control maximum sustainable yield agar weren’t added streptomycin. Establishment of the inoculums strain was estimated as the percentage of isolates (from 20 streaked on agar) which were resistant to streptomycin.

Total genomic DNAs from *Rh. leguminosarum* strains were extracted by using Fast DNA® Kit (MP Biomedicals), according to manufacturer’s instruction. The concentration and the purity of DNA were estimated spectrophotometrically at 260 and 280 nm (Nano Drop ND – 1000). Chromosomal portion of the *Rhizobium leguminosarum* genome was characterized using the automated ribosomal intergenic spacer analysis (ARISA). ARISA distinguishes microbial populations based on the length heterogeneity in the ribosomal intergenic spacer region (Jones et al., 2007). The conditions for ARISA polymerase chain reaction (PCR) using the 1406f/23sr primer set. Primer set 106f/23 Sr consist of 5’ – TGYACACCGCCTTG – 3’ (forward primer sequence) and 5’- GGTTCACCCATCRG – 3’ (reverse primer sequence) methods described by Angela D. Kent (personal communication). A molecular size marker 100 bp + 500 bp DNA Ladder (Fermentas) was run in gels (1%). The restriction patterns were visualized under UV illumination (Cleaver Scientific, DI – HD).
was showed by strain No. 501, but at the pod formation stage by strain No. 110. For the cultivar 'Ada' at the butonisation, anthesis and pod development stages in both soil types the highest resistance to streptomycin was showed by strain No. 408. In both soil types at the butonisation, anthesis and pod development stages the lowest resistance to streptomycin was showed by strain No. 501. The obtained results in cultivar 'Ada' and 'Lielplatone' showed that resistance of the used *Rh. leguminosarum* strains to streptomycin decreases in both soil types from the anthesis stage forward. This is related to decreased activity of *Rh. leguminosarum* strains and nodules agedness.

![Figure 1. Rh. leguminosarum strains resistance to streptomycin at anthesis phase, %:](image)

The field bean cultivar 'Ada' shoot fresh mass was significantly influenced by soil type ($F_{\text{fact}} > F_{\text{crit}}$), *Rh. leguminosarum* strains ($F_{\text{fact}} > F_{\text{crit}}$) and plant development stages ($F_{\text{fact}} > F_{\text{crit}}$) (Fig. 2.). The highest fresh mass was detected at pod development stage, and the highest increase of fresh mass was found from anthesis till pod formation stage. A significant influence of soil type ($F_{\text{fact}} > F_{\text{crit}}$) and plant development stages ($F_{\text{fact}} > F_{\text{crit}}$) on shoot fresh mass of the cultivar 'Lielplatones' was observed. The impact of *Rh. leguminosarum* strains was not observed. The highest fresh mass was detected at pod development stage, and the highest increase of fresh mass for cultivars 'Ada' and 'Lielplatone' cultivated in soil B. The significant impact of cultivar was observed on field bean fresh weight at the pod formation stage ($F_{\text{fact}} > F_{\text{crit}}$).

![Figure 2. The field bean shoot fresh mass at the anthesis phase, g per plants:](image)
Shoot dry matter of field bean cultivar ‘Ada’ was significantly affected by soil type (\(F_{\text{act}} \, 5.015 \geq F_{\text{crit}} \, 3.05\)) and plant development stages (\(F_{\text{act}} \, 81.418 > F_{\text{crit}} \, 2.42\)) (Fig. 3). At the end of vegetation period the highest dry matter was observed in field beans cultivated in soil A with inoculums No. 501 and cultivated in soil B with inoculums No. 110. A significant impact of cultivar was observed only at the pod development stage. A significant impact of soil type (\(F_{\text{act}} \, 14.565 > F_{\text{crit}} \, 3.05\)), and plant development stages (\(F_{\text{act}} \, 20.492 > F_{\text{crit}} \, 2.42\)) on field bean cultivar ‘Lielplatone’ shoot dry matter was observed. At maturity stage the highest dry matter was in the field beans cultivated in soil A with inoculums No. 110 and cultivated in soil B with inoculums No. 408. Results show that dry matter in cultivars ‘Ada’ and ‘Lielplatone’ significantly increased from the pod development stage till maturity stage.

No significant impact of the \(Rh. \, leguminosarum\) strains on total nitrogen amount at the anthesis stage was detected (Fig. 4). For cultivar ‘Ada’ cultivated in both soil types the highest total nitrogen amount at anthesis stage was observed in field beans without inoculants. Whereas for cultivar ‘Lielplatone’ cultivated in soil A the highest total nitrogen amount at anthesis stage was detected in field beans inoculated with strain No. 501 and in soil B in field beans inoculated with strain No. 408. The observed results showed that the highest total nitrogen amount was in the field beans cultivated in soil A. This is explained by soil A containing less organic matter, organic and inorganic nitrogen, and lower microbiological activity as compared to soil B. Less organic matter, organic and inorganic nitrogen induce \(Rh. \, leguminosarum\) strain and indigene nitrogen fixing bacteria to fix \(N_2\) from the atmosphere more actively. Lower microbiological activity causes less competition with other soil microorganisms, although this question demands more research.

The fingerprintings show differences between \(Rh. \, leguminosarum\) strains. The characterizing DNS fragments used primers 106f and 23Sr the \(Rh. \, leguminosarum\) strain base pairs (bp) range from 1500 – 3500 bp.
Conclusions

1. A significant impact of soil type, inoculant and plant development stage on fresh weight of plants was observed.

2. The highest fresh weight, shoot dry matter and pod dry matter was detected in cultivars cultivated in soil with higher microbiological activity. The highest total nitrogen amount was in field beans cultivated in soil with lower soil microbiological activity.

3. *Rh. leguminosarum* strain resistance to streptomycin decreases with the plants development stages from anthesis forward in both soil types and both cultivars.

For the cultivar ‘Lielplatone’ the highest resistance on streptomycin was showed by strain No. 110, but for cultivar ‘Ada’ by strain No. 408.

4. For understanding the role and significance of indigenous nitrogen fixing bacteria in competition with *Rh. leguminosarum* strain, future studies are needed.

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References


