

## Development of symbiotic interactions in the faba bean (*Vicia faba* L.) roots

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**Abstract.** Double-inoculation of faba bean (*Vicia faba* L.) seeds before sowing with rhizobium bacteria and mycorrhizal fungi is a known agronomic practice. It improves symbiotic nitrogen fixation and enhances legume supply with mineral elements and water. Effective symbiosis makes it possible to replace some of the required mineral fertilizer needed for plant growth with symbiotically fixed. This will ensure more environmentally friendly agricultural production. The formation of an effective symbiosis depends on a number of biotic and abiotic factors affecting the biochemical signals released by the partners. The biochemical mediator for symbiosis formation is flavonoids. The aim of this experiment was to test the effects of rhizobial and mycorrhizal inocula on symbiosis formation under different temperatures. Beans are an important source of protein for animal feed and human consumption. Four cultivars of faba beans were used - two *V. faba* var. *minor* Beck. - 'Fuego' and 'Lielplatone', and two *V. faba* var. *major* Harz. - 'Bartek' and 'Karmazyn'. The combination of microorganisms for seed inoculation influenced the frequency of root mycorrhization and abundance of arbuscules. The content of flavonoids in seed exudates correlated ( $r = 0.93$ ) with germination temperatures. The use of mycorrhizal fungi alone or in combination with rhizobia reduced the amount of flavonoids in the bean seed exudate. In the pot experiment the amount and size of nodules significantly differed between cultivars. Use of mycorrhizal preparation mitigated the effect of inadequate germination temperature. Higher degree of mycorrhization and more intense formation of arbuscules formation was observed in the bean roots grown in vegetation pots in comparison with field ones. Local bred 'Lielplatone' had significantly better compatibility with microsymbionts in local agroclimatic conditions.

**Key words:** flavonoids; rhizobia; mycorrhiza; nodule; temperature, faba beans.

### INTRODUCTION

Legumes usually are grown using different soil management practices; seeds are often inoculated before sowing. Sometimes seeds are inoculated with both rhizobia and mycorrhizae fungi. The reason for this double-inoculation is not only to improve the symbiotic nitrogen fixation, but also to enhance the supply of legume with mineral

elements and water. The formation of an effective symbiosis depends on several environmental factors including the interaction of microorganisms in rhizosphere. In the temperate climate zone the optimal environmental requirements of plants and microorganisms must be balanced in order to create an effective symbiosis. In Latvia agroclimatic conditions the faba beans are sown in early spring when soil temperature for microorganism activity usually is not optimal. The average air temperature during this time is between 5 and 10 °C (data obtained from the 'Latvia Environment, Geology and Meteorology Centre, <https://www.meteo.lv/>). Inappropriate temperature and thus slowed seed germination can affect the formation of symbiosis. Optimal root zone temperature for seed germination can vary between faba bean varieties, especially if seeds are inoculated with microorganisms. *Vicia faba* var. *major* seeds require higher germination temperature compared to *Vicia faba* var. *minor* (Senberga et al., 2018). In fact, faba beans are often mentioned as more sensitive to biotic and abiotic factors than other legumes, especially in the case of soil water content and temperature. Soil temperature affects not only the germination rate and the development of the primary root, but can also affect the biochemical processes in the plant. Biochemical mediators must be synthesized for both: the plant and the microorganisms. Only then a symbiotic union can be formed between symbionts (Hauggaard-Nielsen et al., 2011; Šiaudinis et al., 2011).

The biochemical mediator of symbiosis formation is flavonoids, secondary metabolites that develop in plants in low concentration. Flavonoids are synthesized and released in the rhizosphere as a protection mechanism against pathogenic bacteria and fungi. Despite the antifungal, antibacterial and antioxidant properties, flavonoids are recognized as early mediators in the rhizosphere. They exude not only from the roots of the plant, but also from seeds (Maj et al., 2010). Qualitative and quantitative composition of flavonoids depends on used legume species, microorganisms and soil conditions. During seed germination and primary root growth, the composition of flavonoids released in the rhizosphere may differ. A variable accumulation of flavonoids in the plant roots during the formation of symbiosis has also been observed (Guenoune et al., 2001). The flavonoid composition in rhizosphere depends also on the bacterial community. It is especially important during legume – rhizobia symbiosis establishment. Flavonoids released in rhizosphere during symbiosis establishment, are important signal molecules, coordinating the actions of the genes that induce gene transcription in the microbial symbionts, as specific genes essential for the formation of nodule and N<sub>2</sub> fixation (Schultze & Kondorosi, 1998, Day et al., 2001).

The released flavonoids also depend on the legume species and the group of microorganisms. Qualitative and quantitative changes in the composition of flavonoids have been also identified in the mycorrhization process. Comparing the composition of flavonoids accumulated throughout the time of root colonization by mycorrhizae fungi, it was found that coumestrol and medicarpin were accumulated at the beginning of root colonization and at the end of the process. The accumulation levels of ononin and daidzein were higher at the beginning of root colonization, whereas amount of genistein was not changed (Larose et al., 2002). Guenoune et al. (2001) proved that the flavonoid medicarpin accumulated in roots with high phosphate (P) levels. Medicarpin have inhibitory effect on hyphal growth of *G. intraradices*, and is known to possess antifungal activity towards other fungi, thus possibly preventing the roots with a high P concentration from being colonized by arbuscular mycorrhiza fungi (AMF). Flavonoid accumulation in legume roots can be also promoted by rhizobia (Duc et al., 1989).

After the establishment of the symbiotic association, its effectiveness depends on environmental factors. Mycorrhizal growth depends on phosphorus availability, drought stress level and the presence of fungal pathogens (Yang et al., 2015). Plant biological features also influence mycorrhizae growth, however, the effect is poorly investigated and controversially interpreted, especially for the capacity to fix nitrogen and the C-fixation pathway (Yang et al., 2016). Hartnett et al. (1993) found that the effects of arbuscular mycorrhizae fungi can differ at the seedling stage and later development stages of plant.

Factors influencing interaction between symbionts are widely studied. Yang et al. (2015) by meta-data analysis summarised, that arbuscular mycorrhizae fungi can modify plant root morphology and architecture. Results also showed that the type of root system potentially modifies plant growth responds to colonization by AMF. For instance, plants with taproots respond better to AMF than plants with a fibrous root system. But according to Azcón et al. (1991) *Medicago sativa* L. plants with taproot system are highly mycorrhiza dependent, but *Hordeum vulgare* L. with fibrous root system does not show strong positive growth response to colonization by arbuscular mycorrhizal fungi.

Both rhizobia and mycorrhizal fungi receive approximately 4–16% of the photosynthesis products from plant to ensure their activity. In cases where the productivity of photosynthesis is not sufficient to supply all components of the symbiotic system with carbon compounds, they become competitors. In such cases the growth of symbiotic plants may be delayed compared to non-symbiotic plants (Harris et al., 1985, Bethlenfalvay & Newton, 1991).

According to Bruns et al. (2008), understanding the role and effects of mycorrhizal fungi on the nitrogen assimilation, translocation and regulation mechanism in the plant allows a better management of mycorrhizal fungi in sustainable agriculture.

Thus our hypothesis is – in suitable environmental conditions *Rhizobium* bacteria and mycorrhizae fungi may have a beneficial effect during faba beans seeds germination and root system development. The aim of this experiment was to test effects of rhizobia and mycorrhiza fungi inoculum on symbiosis formation under different temperatures. After establishment of effective symbiosis the replacement of some of the required mineral fertilizers for plant growth with symbiotically fixed ones is possible. This will ensure more environmentally friendly agricultural production.

## **MATERIALS AND METHODS**

For the assessment of symbiosis formation, three sets of tests were organized.

Experiment 1 – Laboratory experiment in petri dishes. Determination of the amount of flavonoids in the seed exudate during germination depending on the presence of microsymbionts.

Experiment 2 – Pot experiments. Comparison of inoculation efficiency in greenhouse conditions.

Experiment 3 – Field trials. Comparison of different inoculation variants and determination of the abundance and mycorrhization intensity in faba bean roots.

### **The design of the experiments**

Experiment 1. A laboratory experiment for assessment of seed germination rate and flavonoid content was conducted in Petri dishes at four temperatures, namely 4, 8, 12,

20 °C. Four faba bean (*Vicia faba*) cultivars were used - two *V. faba* var. *minor* Beck. - 'Fuego' (Pflanzenzucht Hans Georg Lembke KG, Germany) and 'Lielplatone' (Institute of Agricultural Resources and Economics, Latvia), and two *V. faba* var. *major* Harz. - 'Bartek' and 'Karmazyn' (Torseed®, Poland) were used. For *V. faba* var. *minor* 10 seeds were used per dish, for *V. faba* var. *major*, 5 seeds per dish. Before inoculation the seed surface was sterilized (30 sec in 70% ethanol and 3 min in 5% sodium hypochlorite). Three seed inoculation variants were chosen: mixture of rhizobia strains (R), mycorrhizae inoculum (M) and double inoculation with rhizobia and mycorrhizae (RM). For control, seeds were germinated without microsymbionts (K). The test was arranged in 4 replications (respectively 40 or 20 seeds per variant). 20 mL of sterile distilled water was added in each petri dish.

Experiment 2. At the end of seed germination experiment in Petri dishes, the experiment was continued as pot experiment. 5 seedlings from each variant were planted in pots. 5 L pots were filled up with non-sterile soil similar with field trial soil in the experiment 3. Temperature regime at night was  $10 \pm 5$  °C and  $18 \pm 5$  °C during the day. At the end of experiment (BBCH 50-53), plants were weighed and the ratio of root and shoot fresh weight was calculated. Fresh and dry weight of nodules, and mycorrhization parameters were established. The abbreviation **BBCH** derives from **B**iologische **B**undesanstalt, **B**undessortenamt and **C**hemical industry. BBCH-scale is a system for a uniform coding of phenologically similar growth stages of all mono- and dicotyledonous plant species (Meier, 2001).

Experiment 3. The field trials were arranged in the experimental plots of the Study and research farm 'Pēterlauki' of Latvia University of Life Sciences and Technologies. The pre-crop of the field trial plots were cereals, and no trials with rhizobia bacteria or mycorrhizal fungi had been carried out in these plots for more than 10 years. According to WRB 2015, the used soil was an Endocalcaric Endoabruptic Luvisol (Aric, Endoclayic, Cutanic, Hypereutric, Ochric, Endoraptic, Anosiltic, Protostagnic, Epiprotovertic). The same seed inoculation variants were used. Size of each plot was 7 per 1.5 m. The faba beans were sown with a seeding-machine. The sowing rate was 45 seeds per m<sup>2</sup>.

### **Microorganisms used for seed inoculation**

*Rhizobium* sp. strains were obtained from the Collection of Rhizobia of the Latvia University of Life Sciences and Technology. Strains RP023 (isolated from *Pisum* sp.) and RV407 (isolated from *Vicia faba*) were mixed and used in the experiment. Both strains were isolated from Latvian soils. Seeds were inoculated before sowing by soaking in the solution with approx. 10<sup>6</sup> bacteria cells per mL.

The inoculum of mycorrhizae was produced by company Symbiom<sup>®</sup>, Czech Republic. *Glomus claroideum*, *Glomus intraradices* and *Glomus mosseae* were present in the inoculum. The mycorrhizal inoculation was performed at sowing. 20 mL of the arbuscular mycorrhiza fungi (AMF) inoculum were placed below each seed.

### **Determination of flavonoid concentration and composition**

After seed germination, water with excreted compounds was collected. Flavonoid concentration was determined according to Robaszkiewicz et al. (2010). Germination solution (2.5 mL) was mixed with 150 µL 5% NaNO<sub>2</sub>. After 5 min incubation at room temperature, 150 µL of 10% AlCl<sub>3</sub> was added, after next 5 min – 1 mL 1 M NaOH.

Absorbance of the solution was read after 15 minutes with spectrophotometer at 415 nm. Flavonoid content was expressed as catechin equivalents (CE) in 100 mL of solution.

For flavonoid composition analysis soaked seeds, primary roots and germinating seed exudate was used. Analyses were performed by HPLC at the Laboratory of Analytical Chemistry, Faculty of Food Technology, Latvia University of Life Sciences and Technology.

Each analysis were made in 4 replications.

### **Assessment of mycorrhizal colonization**

For the assessment of mycorrhizal colonization root fragments were collected. After cleaning them with 10% KOH, root fragments were stained with black Ink (Parker Pen Company, Newhaven, UK) dissolved in 8% acetic acid. Stained root fragments were stored in glycerol:lactic acid:water (v/v/v 1:1:1) solution till microscopic examination (Vierheilig et al., 1998). From each replication 30 root fragments were selected, each approx. 1 cm long. The specimens with the root fragments were examined using a microscope with 10× objective. Parameters of mycorrhizal colonization were evaluated according to the method of Trouvelot et al. (1986) which included mycorrhizal frequency in root fragments, intensity of mycorrhizal colonisation, arbuscules abundance in the root system. The results were calculated by equations incorporated in a computer program MYCOCALC.

Frequency of mycorrhiza in the root system (F%) = (number of fragments with myco/total number)×100.

Intensity of the mycorrhizal colonisation in the root system (M%) =  $(95n_5+70n_4+30n_3+5n_2+n_1)/(\text{total number})$  where  $n_5$  = number of fragments rated 5;  $n_4$  = number of fragments 4 etc.

Intensity of the mycorrhizal colonisation in the root fragments (m%) =  $M \times (\text{total number})/(\text{number with mycorrhizae})$

Arbuscule abundance in mycorrhizal parts of root fragments (a%) =  $(100m_{A3}+50m_{A2}+10m_{A1})/100$  where  $m_{A3}$ ,  $m_{A2}$ ,  $m_{A1}$  are the % of m, rated A3, A2, A1, respectively, with  $m_{A3} = ((95n_5A_3+70n_4A_3+30n_3A_3+5n_2A_3+n_1A_3)/\text{number myco}) \times 100/m$  and the same for A2 and A1.

Arbuscule abundance in the root system (A%) =  $a \times (M/100)$

(<http://www2.dijon.inra.fr/mychintec/Mycocalc-prg/download>).

### **Statistical analysis**

Obtained data were processed using Analysis of Variance (ANOVA) with Microsoft EXCEL software. Differences were considered statistically significant when  $p < 0.05$ . Error bars in figures show the Least Significant Difference (LSD). Correlation coefficient was used to compare flavonoids content, seed germination temperatures and 100 nodule weights.

## **RESULTS AND DISCUSSION**

### **Flavonoids in the seed exudate**

Experiments in Petri dishes showed that the amount of released flavonoids depended on the germination temperature and the inoculants (Table 1). The highest amount of flavonoids in root exudates was detected at 20 °C degrees for all faba bean

varieties and inoculation variants. The amount of flavonoids in seed exudates proves a strong linear positive correlation ( $r = 0.93$ ) with germination temperatures. The highest flavonoids content in exudates was found in variants where cv ‘Fuego’ was germinated.

**Table 1.** Mean amount of flavonoids (mg CE mL<sup>-1</sup>) in seed exudate during faba bean seed germination

Germination temperature, °C	Inoculation variants	<i>Vicia faba</i> var. <i>major</i>		<i>Vicia faba</i> var. <i>minor</i>	
		Karmazyn	Bartek	Fuego	Lielplatone
4	K	0.202 <sup>b</sup>	0.165 <sup>b</sup>	0.314 <sup>a</sup>	0.264 <sup>b</sup>
	R	0.212 <sup>b</sup>	0.125 <sup>a</sup>	0.347 <sup>a</sup>	0.263 <sup>b</sup>
	M	0.189 <sup>a</sup>	0.122 <sup>a</sup>	0.326 <sup>a</sup>	0.261 <sup>b</sup>
	RM	0.180 <sup>a</sup>	0.202 <sup>c</sup>	0.304 <sup>a</sup>	0.170 <sup>a</sup>
8	K	0.202 <sup>a</sup>	0.154 <sup>a</sup>	0.378 <sup>b</sup>	0.260 <sup>b</sup>
	R	0.208 <sup>a</sup>	0.168 <sup>a</sup>	0.371 <sup>b</sup>	0.253 <sup>b</sup>
	M	0.214 <sup>a</sup>	0.227 <sup>b</sup>	0.306 <sup>a</sup>	0.313 <sup>c</sup>
	RM	0.230 <sup>b</sup>	0.155 <sup>a</sup>	0.359 <sup>b</sup>	0.225 <sup>a</sup>
12	K	0.305 <sup>c</sup>	0.215 <sup>b</sup>	0.466 <sup>b</sup>	0.337 <sup>b</sup>
	R	0.224 <sup>b</sup>	0.228 <sup>b</sup>	0.408 <sup>a</sup>	0.273 <sup>a</sup>
	M	0.161 <sup>a</sup>	0.161 <sup>a</sup>	0.417 <sup>ab</sup>	0.273 <sup>a</sup>
	RM	0.169 <sup>a</sup>	0.204 <sup>b</sup>	0.446 <sup>ab</sup>	0.254 <sup>a</sup>
20	K	0.441 <sup>c</sup>	0.341 <sup>c</sup>	0.644 <sup>a</sup>	0.444 <sup>b</sup>
	R	0.348 <sup>a</sup>	0.329 <sup>c</sup>	0.704 <sup>b</sup>	0.371 <sup>a</sup>
	M	0.390 <sup>b</sup>	0.285 <sup>b</sup>	0.760 <sup>c</sup>	0.359 <sup>a</sup>
	RM	0.421 <sup>c</sup>	0.237 <sup>a</sup>	0.690 <sup>b</sup>	0.384 <sup>a</sup>

Values in the same column and same germination temperature with identical letters are not significantly different ( $p > 0.05$ ), LSD 0.075. Variants K – control; R – inoculation with rhizobia strains; M – inoculation with mycorrhiza preparation; RM – inoculation with rhizobia strains and mycorrhiza preparation.

The use of microsymbionts influenced the release of flavonoids. At 77.7% occasions, the use of the microsymbiont(s) significantly lowered the amount of released flavonoids. The largest effect was observed with mycorrhizal fungi preparation (M), then followed with double inoculation (RM) (Table 1). In variants where rhizobia were used, only in 9.4% occasions significant ( $p < 0.05$ ) differences were observed. Significant decrease of the amount of flavonoids for *Vicia faba* var. *major* cultivars was detected at 12 °C.

The composition of the flavonoids differed in germinating seed, primary root and exudate. The main flavonoid in the primary roots was catechin, but rutin and luteolin were found at a lower concentration. Seed exudates contain higher concentration of catechin and rutin, but the amount of luteolin comparing to primary roots was decreased. Quercetin was detected in seed and primary root, but not in the exudate.

Experiments have shown that root development in inadequate temperature can be delayed (Senberga et al., 2018). Inadequate temperature may influence the amount and composition of flavonoids. Plants release flavonoids from small roots and root hairs, forming biochemical signals, so that hyphae grow directly on the roots. Microorganisms in the rhizosphere often induce increased release of flavonoids, thus facilitating root colonization (Frey-Klett et al., 2007). The amount of released or accumulated flavonoids depends not only on the presence of rhizobia or mycorrhizal fungi, but also on the

population of microorganisms in the rhizosphere that may affect biochemical processes and the formation of nodules, and the intensity of colonization of mycorrhiza.

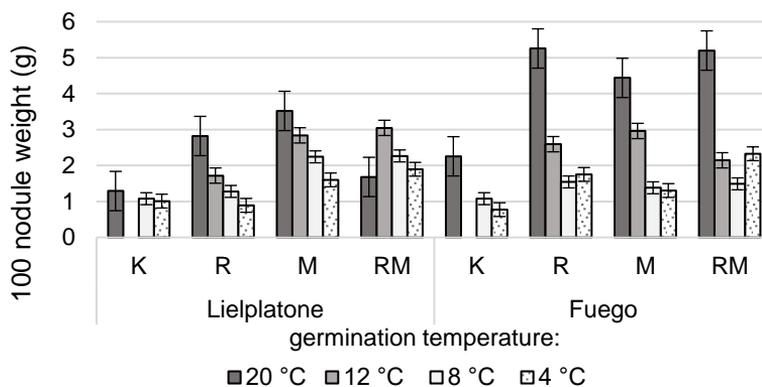
Various studies (Larose et al., 2002; Petrusa et al., 2013; Wang et al., 2015) have confirmed that temperature and soil moisture content has significant effects on the flavonoid biosynthesis and expression not only in root exudate, but in different parts of the plant. Decrease of released flavonoids depended on plant variety as well as on inocula composition. Flavonoid content in the seed exudate of variety 'Bartek' and 'Karmazyn' decreased at the temperature 12 °C when inoculated with mycorrhizal fungi. This may also be due to the accumulation of flavonoids in the roots. It can influence the released amounts of flavonoids in rhizosphere. Larose et al. (2002) determined that roots colonized by mycorrhizae fungi accumulate different amount of flavonoids. In these experiments also the accumulation levels varied depending on the root-colonizing fungi. For example, flavonoid coumestrol was accumulated more in roots colonized by *G. intraradices*, but less in roots colonized by *G. mosseae*. Similarly, daidzein was more accumulated in roots colonized by *G. intraradices* and *G. mosseae*, but detected less in roots colonized by *G. rosea*. However, the statement of Schmidt et al. (1994), that flavonoid accumulation can be enhanced by rhizobia as well, was confirmed by our results.

#### **Germinated faba bean growth at vegetation pot trial**

At the end of the vegetation pot trial no statistically significant differences ( $p > 0.05$ ) were detected for the fresh weight of plant shoots and roots. Obtained data showed only a tendency that inoculated plants can form larger aboveground part. The largest ratio of root to shoot weight was detected in germination variants with mycorrhizae fungi inoculation. In this inoculation variant average root: shoot ratio was 0.60 and varied for different cultivars between 0.49 and 0.71. The lowest ratio was in the version where seeds were inoculated only with rhizobia. Here the average ratio was 0.48, and varied between 0.42 (cv 'Bartek') and 0.53 (cv 'Lielplatone'). The largest root system was observed for cv 'Bartek' with mycorrhiza inoculation. Inoculation with only rhizobia reduced root growth in most cases.

In vegetative pot experiment, only some nodules formed on the roots of large-seeded beans (cv 'Bartek' and 'Karmazyn') and no significant differences were observed between different inoculation variants of these cultivars. *V. faba* var. *minor* 'Fuego' and 'Lielplatone' formed enough nodules and that allowed to assess effect of inoculation. The results showed that lower temperature during germination influenced nodule formation (Fig. 1). Whereas plants were grown in non-sterile soil, some nodule formation was also observed in samples grown without rhizobia inoculum (K and M). Indigenous rhizobia formed smaller nodules compared to the variants with rhizobia inoculation, especially on those plant roots which seeds were germinated in lower temperature.

Faba bean seed inoculation only with *Rhizobium* bacteria is not always a sufficiently effective agricultural practice, especially when seeds are sown in early spring, when the root zone temperature does not exceed 4 °C (Senberga et al., 2018). Though bacteria from *Rhizobiaceae* are able to survive at 4 °C (Drouin et al., 2000) and bacterial activity increases with increasing temperature, prolonged exposure of inoculated seeds to low temperatures can decrease the ability to develop symbiosis (Fyson & Sprent, 1982).



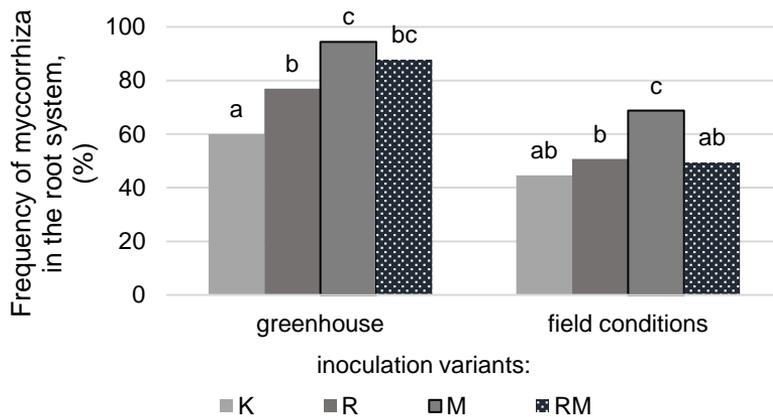
**Figure 1.** 100 nodule weight at the end of vegetation pot experiment: K – control; R – inoculation with rhizobia strains; M – inoculation with mycorrhiza preparation; RM – inoculation with rhizobia strains and mycorrhiza preparation.

The effect of germination temperature on nodule weight was observed also in the vegetation pot experiments and depended on cultivar. Nodules on the roots of ‘Fuego’ germinated in 20 °C was twice heavier as ‘Lielplatone’ ones. Such a difference may arise due to the biological nature of the varieties and the suitability for the specific agroclimatic conditions. Legume seed inoculation with mycorrhizae fungi alone or together with *Rhizobium* showed positive influence on nodule formation compared to control samples. According to the results, the decrease of released flavonoids at a low temperature and reduced root growth could have led to a formation of smaller, less effective nodules. This is consistent with the statement of Nap & Bisseling (1990) and Hirsch (1992), that the size of the nodule is related to their functional activity. Heavier nodules are considered to be more effective in N<sub>2</sub> fixation.

A correlation was detected between germination temperature and flavonoids exudation ( $r = 0.95$ ), and nodule weight and flavonoid exudation ( $r = 0.77$ ) for cultivar ‘Fuego’. For cultivar ‘Lielplatone’ a significant correlation ( $r = 0.83$ ) was detected only between flavonoid exudation and temperature.

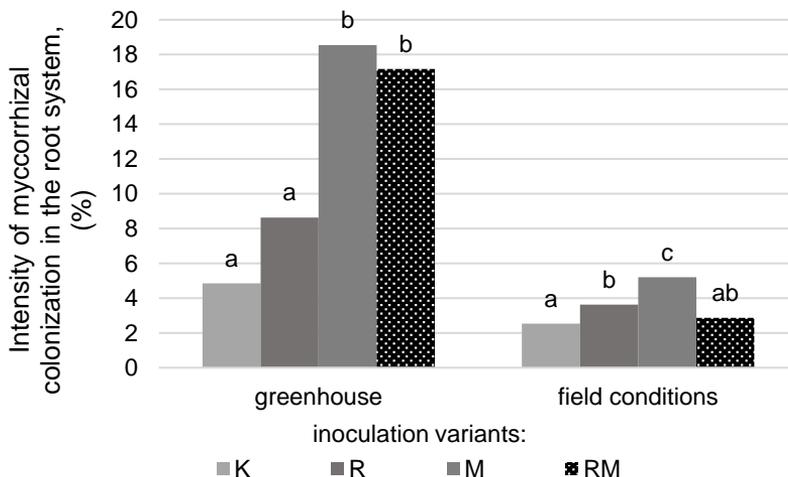
### Abundance of mycorrhizae fungi in faba beans root

The effect of growth conditions on the frequency and intensity of mycorrhizal colonization was observed comparing vegetation pot and field trials. Frequency of mycorrhiza fungi (Fig. 2) on inoculated faba bean roots significantly differed ( $p < 0.05$ ) between greenhouse experiment and field trials. In the field trials, plants and microorganisms were more susceptible to fluctuating environmental conditions, which is not always favourable for the formation of symbiosis. In the field cultivated faba bean roots mycorrhizae fungi structures were observed less, compared to samples grown in vegetation pots in greenhouse. Significant differences between control and inoculated variants were detected in the vegetation pot experiment, the most distinct in samples with mycorrhizae fungi inoculum. Plants inoculated with mycorrhiza fungi show different root colonization possibilities compared to samples without inoculation due to the composition of indigenous fungi species. Yang et al. (2015) concluded that plants with taproot are more responsive to symbiosis formation if AMF species richness in rhizosphere is low.



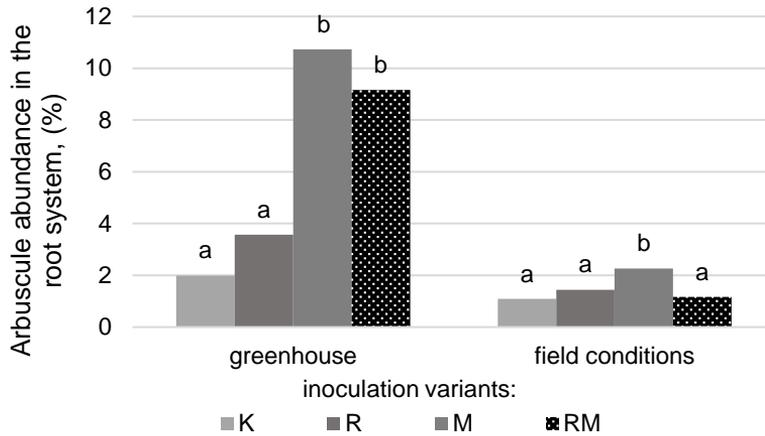
**Figure 2.** Comparison of the frequency of mycorrhiza fungi in the root system of cv 'Bartek' in vegetation pot experiment and in field trial. Means designated with the same letter are not significantly different ( $p \leq 0.05$ ): K – control; R – inoculation with rhizobia strains; M – inoculation with mycorrhiza preparation; RM – inoculation with rhizobia strains and mycorrhiza preparation.

Since non-sterile soil was used in vegetation pot trial, structures of the fungus was found also in control. In vegetation pots the root colonization with mycorrhizae fungi structures was in average 3.6 times intense than in the control variant (Fig. 3). In variants where seeds were inoculated only with rhizobia, mycorrhizal colonization intensity exceeded control variants averagely 1.8 times. For samples grown on field, differences between variants inoculated only with rhizobia and control were averagely 1.3 times.

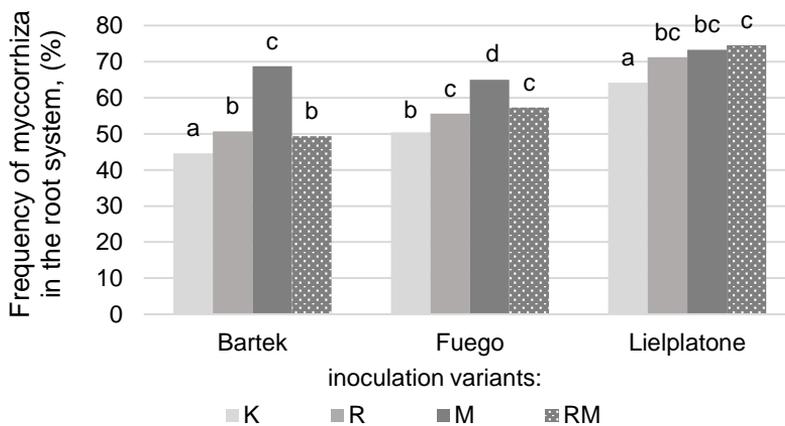


**Figure 3.** Comparison of the intensity of mycorrhiza fungi in the root system of cv 'Bartek' in vegetation pot experiment and in field trial. Means designated with the same letter are not significantly different ( $p > 0.05$ ): K – control; R – inoculation with rhizobia strains; M – inoculation with mycorrhiza preparation; RM – inoculation with rhizobia strains and mycorrhiza preparation.

In vegetation pot experiment arbuscules abundance in faba beans root system showed significant ( $p < 0.05$ ) difference between inoculation variants (Fig. 4). The arbuscules abundance in root fragments of inoculated variants (M, RM) was 5.4, and 4.6 times higher than in control variants. Less significant differences were observed for samples grown at the field conditions. Significantly higher amount of arbuscules in roots was in the variants with mycorrhizae inoculum grown in greenhouse conditions. In contrast the field conditions did not increase abundance of arbuscules.



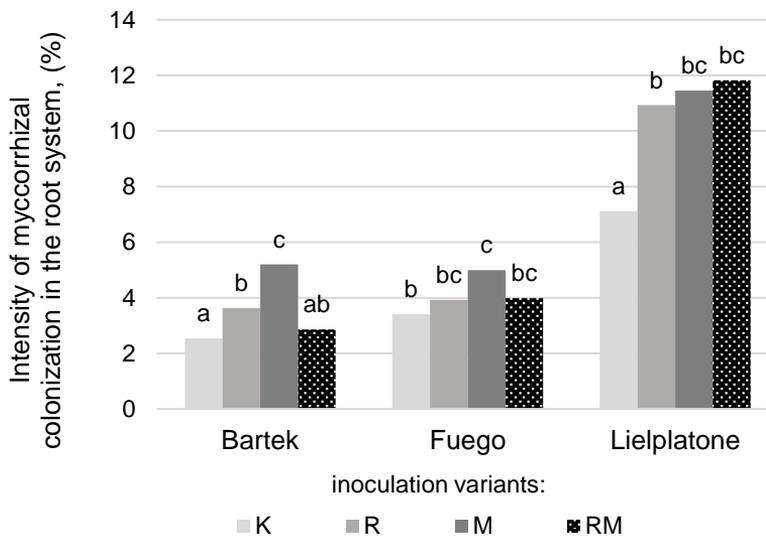
**Figure 4.** Comparison of arbuscules abundance in the root system of cv 'Bartek' in vegetation pot experiment and in field trial. Means designated with the same letter are not significantly different within growing conditions ( $p > 0.05$ ): K – control; R – inoculation with rhizobia strains; M – inoculation with mycorrhiza preparation; RM – inoculation with rhizobia strains and mycorrhiza preparation.



**Figure 5.** Frequency of mycorrhiza structures in faba bean root system in field experiment. Means designated with the same letter are not significantly different within variety ( $p > 0.05$ ): K – control; R – inoculation with rhizobia strains; M – inoculation with mycorrhiza preparation; RM – inoculation with rhizobia strains and mycorrhiza preparation.

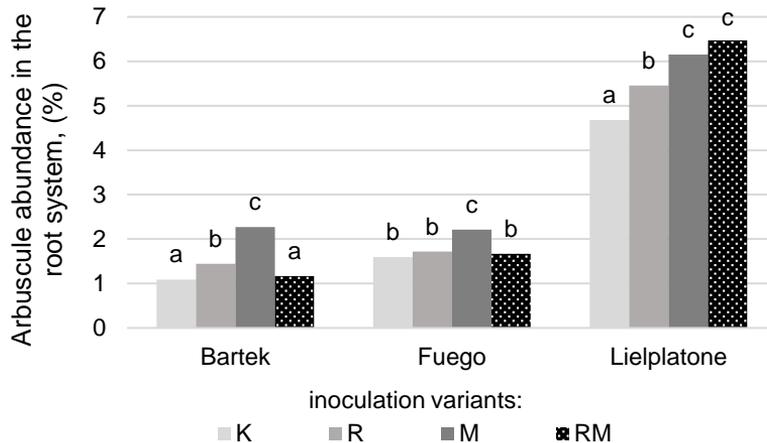
Observed differences between cultivars in field conditions are shown in Fig. 5. Frequency of mycorrhiza structures in all cultivars root systems were higher in inoculated variants in comparison with control, but only in cultivar ‘Lielplatone’ more fungi structure were observed in double inoculated variant compared to single inoculated samples. The degree of mycorrhization in field trials was significantly lower than in pot experiments. This can be explained by the weather conditions and their influence on plant growth, and the manifested competition of microorganisms in soil rhizosphere. Similar results are reported by Gamalero et al. (2002), where modifications of root morphogenesis and growth and mycorrhization depended on plant growth conditions and soil fertility.

Similar results between variants grown on the field were obtained for intensity of mycorrhizal colonisation (Fig. 6). For cultivar ‘Lielplatone’ more significant differences were determined between control and all three inoculation variants. If frequency of mycorrhizal structure did not show significant difference between cultivars, then mycorrhizal colonization intensity was significantly higher in cv ‘Lielplatone’ root system. It could be explained by the fact that this cultivar was bred in Latvia and is more suitable for local agroclimatic conditions. Thus, more appropriate conditions could create more favourable conditions for mycorrhizae fungi spreading in the roots.



**Figure 6.** Intensity of mycorrhizal colonisation in the root system in faba bean root system in field experiment. Means designated with the same letter are not significantly different within variety ( $p > 0.05$ ): K – control; R – inoculation with rhizobia strains; M – inoculation with mycorrhiza preparation; RM – inoculation with rhizobia strains and mycorrhiza preparation.

Abundance of arbuscules in the faba beans root were higher in cv ‘Lielplatone’, in comparison with cv ‘Fuego’ and ‘Bartek’ (Fig. 7). Only cv ‘Lielplatone’ in variants with double inoculation formed significantly higher amount of arbuscules in comparison with control.



**Figure 7.** Comparison of arbuscules abundance in the root system of faba bean root system in field experiment. Means designated with the same letter are not significantly different within variety ( $p > 0.05$ ): K – control; R – inoculation with rhizobia strains; M – inoculation with mycorrhiza preparation; RM – inoculation with rhizobia strains and mycorrhiza preparation.

Higher amount of arbuscules forms a wider range of possibilities for the exchange of nutrients between symbionts, thus the formation of the effective symbiosis, and that can result in the increase of bean yield and improve its quality.

## CONCLUSIONS

1. The release of flavonoids from the roots was affected by the seed germination temperature and the microsymbionts used for inoculation. The correlation coefficient between temperature and released flavonoids was  $r = 0.93$ . The inoculation of seeds with mycorrhizal fungi resulted in a decrease in the amount of flavonoids in the exudate. The largest impact of mycorrhization was observed in 12 °C.

2. The amount and size of nodules in the pot experiment significantly differed between *V. faba* var. *minor* and *V. faba* var. *major* cultivars. The effect of germination temperature on vegetative growth of the bean was found. Use of mycorrhiza preparation mitigated the effect of inadequate germination temperature.

3. Higher degree of mycorrhization and more intense formation of arbuscules was observed in the bean roots grown in the pots in comparison with field ones. Degree of mycorrhization depended on used cultivar. Local bred ‘Lielplatone’ was significantly better responsive to microsymbionts in local agroclimatic conditions in comparison with other cultivars used in the trial.

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