Evaluation of effectiveness of *Rhizobium leguminosarum* strains on broad beans

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Abstract

Broad beans are grown worldwide as a source of protein in human diets, forage crop for animals, and for increasing nitrogen content in the soil. Experiments were carried out to investigate the effectiveness of four *Rhizobium leguminosarum* strains from the collection of Latvia University of Agriculture. Two cultivars of broad beans 'Bartek' and 'Karmazyn' were used. Experiments were located in three places with different soil mineral and chemical composition and pH. Streptomicine resistant strains Nr. 23, 110, 407 and 505 were used. Seeds were treated with suspension of bacteria before sowing. Control was without treatment. Plant weight, dry matter and protein content in plants and seeds were analyzed at the flowering stage and at the end of experiment. During flowering stage number of nodules was evaluated. Experiments were done in four replications with 10 plants in each replicate. Results showed that strain efficiency depended on interaction between strain, soil conditions and cultivar. In average higher number of nodules was observed in the roots of 'Karmazyn', but it doesn't reflect on yield. Higher yields, dry mater and protein content in seeds were observed for 'Bartek'. Correlation between protein content and soil fertility was observed in different locations. Strain Nr. 407 promoted protein accumulation in the seeds of both cultivars.

Keywords: *Vicia faba*, protein, rhizobia strains, yield

INTRODUCTION

Broad bean (*Vicia faba* L.) is a cool season grain legume used as a source of protein in human diets, as a forage crop for animals and for increasing nitrogen content in the soil (Duc et al., 2010). Broad beans are grown worldwide, in countries of Europe, North Africa and West Asia. Broad beans are source of organic nitrogen, due to their symbiosis with *Rhizobium*. Therefore, they require less to none nitrogen fertilizers, indicating broad bean as one of crop rotation component in sustainable agriculture (Smil, 2001; Jensen et al., 2010). Broad bean cultivation is increasing, as growers appreciate its benefits to the fertility and health of soils (Paull et al., 2011; Flores et al., 2013).

Broad beans as legumes derive nitrogen from two different sources: taking from the soil, and symbiotically fixing atmospheric nitrogen (N_2) by participation of nitrogen fixing bacteria making nodules on the plant roots. The genetic variability of root and nodule establishment affects acquisition of nitrogen and seed protein content. Furthermore, these parameters are related to the variability in preference for rhizobial genotypes (Bourion et al., 2007). The significance of applying N_2 fixing bacteria to legume seeds at sowing was shown in experiment with soybean plants where the nitrogen-fixing plants were less affected by salt stress than nitrate-fed plants (Kirova et al., 2013). That means that the N_2 fixing bacteria could be used not only to enhance yield (green forage and seeds), but either enable growing legumes in soils with natural salinity or where soils are polluted by protracted fertilizing with mineral fertilizers. Most of stress factors such as salt, heat, and acid stresses supress the growth and symbiotic characteristics of most rhizobia, but there are several strains more tolerant to stress effects (Zahran, 1999). Different *Rhizobium* strains vary in tolerance to fluctuations between temperature and moisture extremes in soil (Boonkerd and Weaver, 1982).

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Field legume inoculation with *Rhizobium* spp. sometimes may be unsuccessful due to the in the soil of native strains that compete with the introduced strain in nodule formation on the host plants (Toro, 1996). Survival of added rhizobia over years in the soil where inoculated plants have been grown depends on the rhizobia strain used, and on the soil (Albareda et al., 2009).

Inoculation with effective rhizobia strains leads to increase of protein content in the plants. The seeds of broad beans averagely contain 25-30% crude protein, 40-50% carbohydrates and 10-15% crude lipids (Macarulla et al., 2001). In addition, recent advances in the genetic improvement of broad bean cultivars have led to the development of a high-yielding line and low tannin cultivars of greater consideration for use in human food and domestic animal feeds (Duc et al., 1999; Gutierrez et al., 2004).

The objective of this study was to investigate the effectiveness of *Rhizobium leguminosarum* strains on the broad beans yield, its formation and quality in Latvia agro-meteorological conditions.

MATERIALS AND METHODS

Investigations were carried out at the Latvia University of Agriculture, Institute of Soil and Plant Sciences in 2013 and 2014. Experiments were located at three places with different mineral and chemical composition of the soil. Soil characteristics of locations are following (mg L^{-1}): field I - peat (N-P-K 62-959-155, pH KCl 6.97 and EC 0.67 mS cm^{-1}), field L- sandy soil (N-P-K 94-916-170, pH KCl 6.69 and EC 0.38 mS cm^{-1}), field M - loamy sand (N-P-K 78-523-170, pH KCl 7.62 and EC 0.6 mS cm^{-1}).

Broad bean cultivars 'Bartek' and 'Karmazyn' were used in experiments.

Streptomicine resistant strains Nr. 23, 110, 407 and 505 from *Rhizobium* Strain Collection of Latvia University of Agriculture were used. Seeds were treated for 30 min with suspension of bacteria before sowing. Concentration of bacteria in suspension was 10^8-10^9 CFU mL^{-1}. Control represented plants without treatment. Experiments were done in four replications with 10 plants in each replicate.

Plant weight, dry matter and protein content were analyzed at the flowering stage and at the end of experiment. Protein content was determined by Kjeldal method. During flowering stage number of nodules was evaluated.

The average of relative air humidity, air temperature and atmospheric pressure for a date was calculated from daily meteorological observations (average from every 24 h) in Latvia, in the nearest station to experiment location.

The obtained data was analysed with multiple factor analysis included in software R studio. Significance level of $p \leq 0.05$ was used.

RESULTS AND DISCUSSION

The production of biomass and dry weight in plants is associated with nitrogen uptake and metabolism. Since adding rhizobia usually results in symbiotic uptake of atmospheric N_2 additional to nitrogen compounds from the soil, the measuring up of biomass and dry mass can be used for evaluating effect of added rhizobia strains. Our results showed that all the used strains promoted increase of biomass, at least in tendencies if not always statistically significant difference. Some strains were more successful than other, particularly Nr 23, 110 and 505 with broad bean cultivar 'Bartek'. Using multiple factor analysis in calculations, we it was found out that there were complex relations between rhizobia strain, chosen cultivar, and soil conditions. Choice of cultivar and soil conditions was essential for effect of rhizobia strain on increasing plant biomass (Figures 1-4). These results are in accordance with a theory that N_2 fixation is related to the variability in preference for rhizobial genotypes (Bourion et al., 2007).

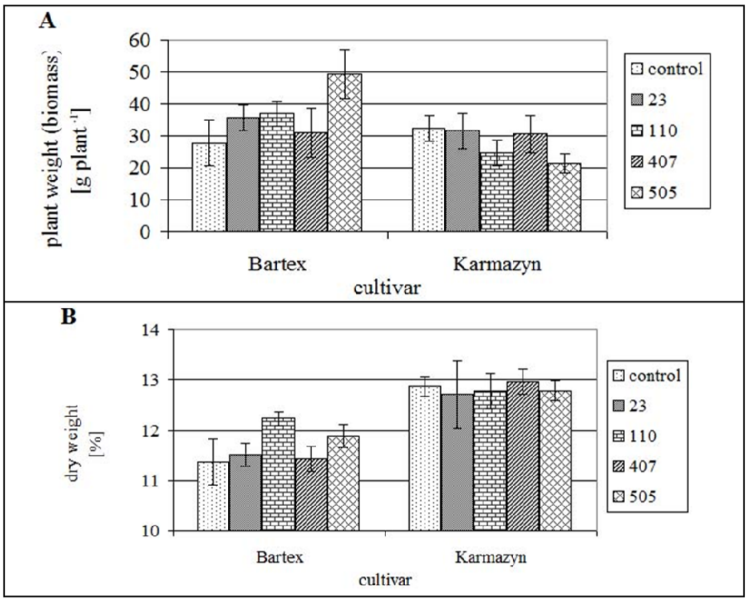

Figure 1. Changes in broad bean plant weight (biomass) (A) and dry weight (B) by preliminary seed tretment with different strains of *Rhizobium leguminosarum*. Comparission of effect on cultivars 'Bartek' and 'Karmazyn'.

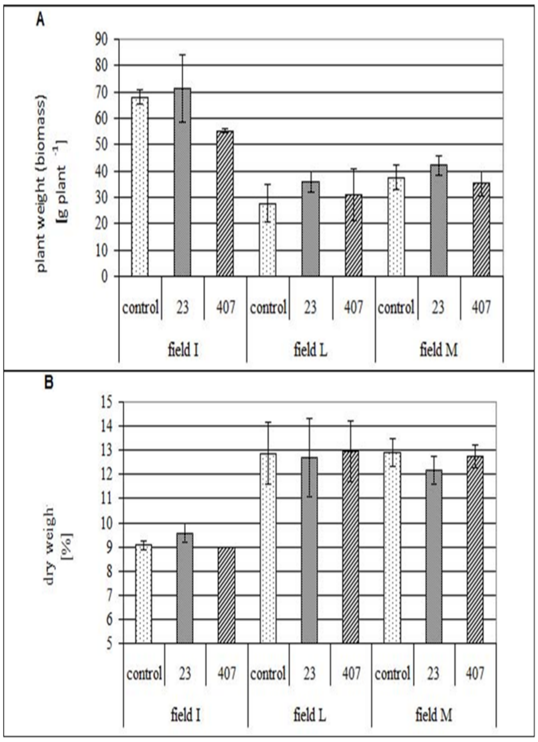

Figure 2. Changes in broad bean cultivar 'Bartek' plant weight (biomass) (A) and dry weight (B) by preliminary seed treatment with different strains of *Rhizobium leguminosarum*, observed in three places (fields: I, L and M) with different soil mineral and chemical composition, and pH. Field I: N-P-K 62-959-155, pH KCl 6.97 and EC 0.67 mS cm^{-1}; Field L: N-P-K 94-916-170, pH KCl 6.69 and EC 0.38 mS cm^{-1}; Field M: N-P-K 78-523-170, pH KCl 7.62 and EC 0.6 mS $cm{-}^{1}$.

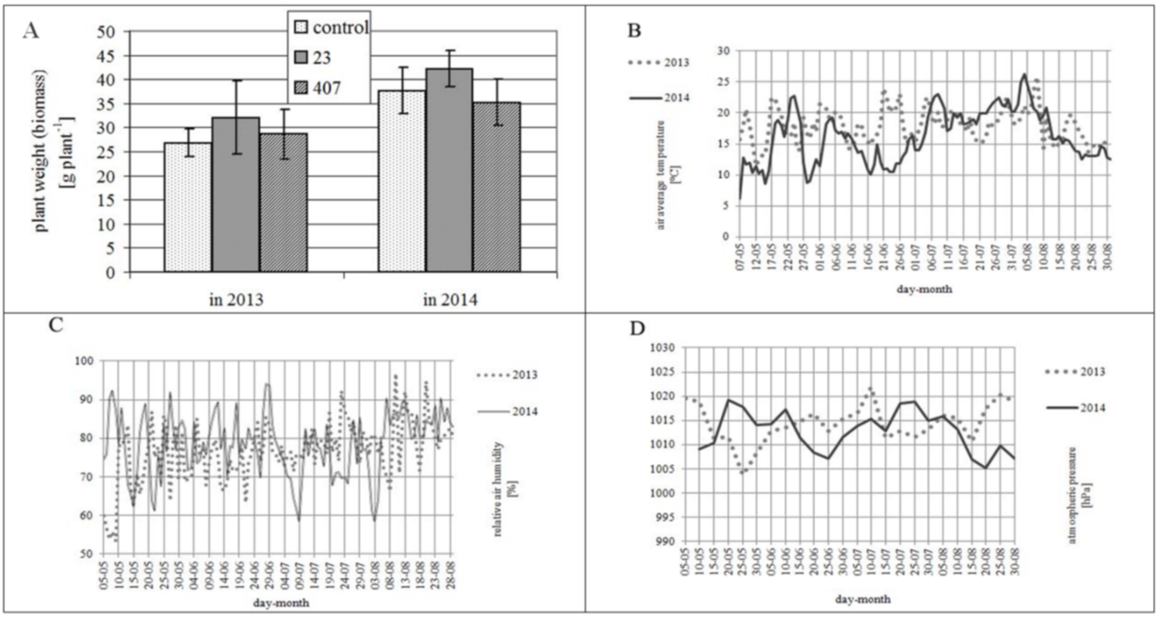

Figure 3. Changes in plant weight (biomass) of broad bean cultivar 'Bartek' plants (A), grown in the same soil, inoculated with different *Rhizobium leguminosarum* strains before sowing, in comparison with changes in average air temperature (B), relative air humidity (C), and atmospheric pressure (D) over growing periods in two years.

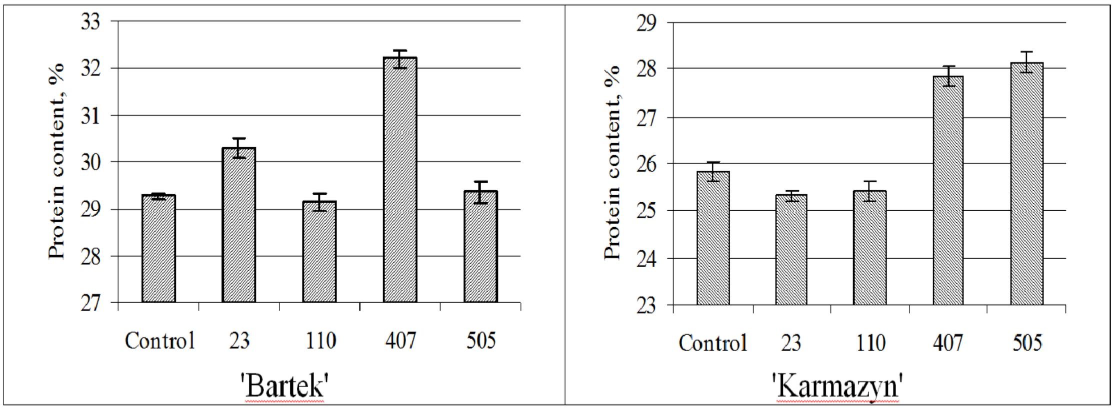

Figure 4. Protein content in the seeds of broad bean 'Karmazyn' and 'Bartek', % in dry matter.

Some strains, as Nr. 407, were not always successful in all soils (Figure 2). The cause might be that the native strains in the soil compete with the introduced strain in nodule formation on the host plants (Toro, 1996). Survival of added rhizobia depends on the soil, too (Albareda et al., 2009).

The experiments were provided in three different places to find out whether the rhizobia effect on beans depends on soil conditions. Using multifactor analysis for data calculations we it was found that the soil conditions were more essential for effect of rhizobia than chosen cultivar. The tendencies of changes in plant biomass were similar in all three places with different soil however the numeric value of biomass were higher and dry mass lower in the field I, where electrical conductivity (EC) was the higher (Figure 2).

In experiment with soybean plants, the nitrogen-fixing plants were less affected by salt stress than nitrate-fed plants (Kirova et al., 2013). In our experiments there could not be discourse about too high salinity as the EC of different soils was 0.38-0.67 mS cm^{-1}. Although our evaluated strains could be examined for ability to enable growing legumes in soils with higher salinity as they were successful in soils with different mineral and chemical composition, and pH (Figure 2).

It is known that some rhizobia strains are more tolerant to stress effects of soil conditions and climate (Zahran, 1999; Boonkerd and Weaver, 1982). The strain Nr. 23 promoted considerable increase of biomass in diverse climatic conditions in two years, one of them with wide temperature and moisture extremes (Figure 3). Positive effect was found in three different soil conditions.

In average higher number of nodules was observed in the roots of 'Karmazyn', but it doesn't reflect on yield.

Two similar experiments in the same soil were provided in different years to find out whether the rhizobia effect on beans depends on climatic conditions. Changes of air average temperature, air relative humidity and atmospheric pressure were observed over both vegetation periods. Broad bean 'Bartek' maintained tendency to be higher in biomass with rhizobia strain 23 at both of experiments although all the observed climatic conditions varied over years at the same dates (Figure 3).

In experiments with inoculation of *Glycine max* cultivar with rhizobia, the biomass, dry weight and other growth parameters increased significantly with the increasing root temperature and low relative humidity of air (Stoyanova, 1996). In open field where the air relative humidity cannot be controlled and maintained invariable, opposite results were found: in the summer with higher air relative humidity, more extremes and lower air average temperature until the flowering stage of plants, the biomass was higher than in the other summer with lower air relative humidity and higher temperature (Figure 3). These results could be explained by the increased soil moisture that resulted in higher biomass of plants. There were no significant differences in dry weight over variants.

Protein content in plants at flowering stage depended on soil type (Table 1). Averagely in soil M cultivars 'Bartek' protein content was 24.2±0.8% of dry matter (dm), but for 'Bartek' at L soil – 19±1% dm, but for I type of soil – 24±2% dm.

Table 1. Protein content in plants at flowering stage, % in dry matter.

Variants	M	L		I	
	Bartek	Bartek	Karmazin	Bartek	Karmazin
Control	24.50±0.10	19.11±0.14	17.94±0.12	21.17±0.14	23.57±0.09
23	23.26±0.12	21.61±0.15	19.75±0.12	24.82±0.17	27.18±0.13
110	-	18.34±0.16	20.12±0.10	26.35±0.18	26.48±0.17
407	24.87±0.14	17.57±0.88	18.07±0.13	27.24±0.19	26.62±0.22
505	-	18.84±0.54	19.32±0.13	23.82±0.14	26.46±0.18

For 'Karmazyn' in soil type L, average protein content was 19±2% dm, but in I type of soil 26±1% dm. Obtained results confirmed that effectiveness of bacteria depends on soil type. In soils M and L *Rhizobium* strains has averagely less effect (accumulated protein), than on plants grown in I type soil, where average protein content is higher and deference between strains is larger. Still mathematical analysis shows significant (α=0.05) differences between strains in one soil type.

Protein content (Figure 4) in 'Karmazyn' was 25.8±0.2% dm for control sample, but the highest protein content 28.2±0.1% was in samples grown with strain 505. Significant differences (p=0.001; α=0.05) were found. On the contrary, strains 23 and 110 had less protein content than control by nearly 2%. Protein content in 'Bartek' was 29.3±0.1% dm for control sample, but the highest protein content 32.2±0.2% was in samples grown with strain 407. Significant differences (p=0.0001; α=0.05) were found. Strain 23 had 30.3±0.3% of protein content, but strain 110 and 505 had not significantly less or more protein that control sample.

CONCLUSIONS

Strain efficiency depended on interaction between strain, soil conditions and cultivar. In average higher number of nodules was observed in the roots of 'Karmazyn', but it doesn't reflect on yield. Higher yields, dry mater and protein content in seeds were observed for 'Bartek'. Correlation between protein content and soil fertility was observed in different locations. Strain Nr. 407 promoted protein accumulation in the seeds of both cultivars.

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