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THE EVALUATION OF EFFECTIVENESS OF *RHIZOBIUM LUPINI* STRAINS

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Abstract

Biologically fixed nitrogen plays an important role in crop rotation of green agriculture. Significant increase of legumes yields are obtained by seed treatment with active nitrogen fixing bacteria strains before sowing. The possibility of the improvement of plant biomass yields through the use of more efficient combinations of *Rhizobia* and legumes was explored.

Experiments were carried out the at Institute of Plant and Soil Sciences Latvian University of Agriculture to investigate the effectiveness of two *Rhizobium lupini* strains- a commercial preparation, *nitragin*, marketed by the company 'Bioefekts' and the strain *Rhizobium lupini* from the collection of the Institute of Soil and Plant Sciences.

The test samples were placed in 5 L pots with washed river sand, mixed with Kemira GrowHow NPK 0 – 5 – 20 and microelements fertilizer. Nitrogen was added as ammonium nitrate, 0.024 g per 1 kg of sand for inoculated plants, and ten times more (0.24 g kg^{-1}) for the control variant, where plants were not inoculated. In the vegetation pot experiments three lupines species, *L. luteus* variety 'Juno', *L. angustifolius* variety 'Sonet' and *L. albus* variety 'Wat' were used. Before sowing the lupine seeds were inoculated with *Rhizobium lupini* strains.

The results showed that plants receiving increased nitrogen dosages sprout later and lag in height in the period of active growth. The plant length depends on plant species. The smallest length was observed of the species *L. angustifolius* variety 'Sonet', the largest are plants of the species *L. luteus* variety 'Juno'. The inoculation of plant seeds promotes increase in plant length till the phase of bud formation. There are no significant differences between *Rhizobium lupini* strains. The largest fresh and dry weights were observed for *L. albus* variety 'Wat', but species *L. angustifolius* had the smallest fresh and dry weight. The largest dry matter content in plants till the stage of bud formation was observed for inoculated plants. After the phase of flowering it was found that symbiotic systems are not sufficient in providing nitrogen. The effectiveness of *Rhizobium lupini* strains are species dependent.

Key words

Rhizobium lupini strains, lupine, inoculation

Introduction

One of the properties of nitrogen fixing bacteria is to form nodules on plant roots and fix nitrogen from the atmosphere. The symbiosis between bacteria of the genus *Rhizobium* and their leguminous host plants results in the formation of root nodules in a species- specific way, in that a particular bacterial species can nodulate only a limited number of host species. Symbiosis converts the inert form of nitrogen (N_2) to organic nitrogen and incorporates it into proteins, nucleic acids and other cellular components. By symbiosis plants provide bacteria with nutrition elements, mostly sugars, and ensure favorable conditions for life in the nodules. Much more viable bacteria returns to the soil after the decomposition of the plants. (Spaink et al., 1987; Stephens and Rask, 2000, Ovalle et al., 2006)

The encouragement of *Rhizobium* - legume symbioses definitely increases the incorporation of biological fixed nitrogen into the soil ecosystem (Metting F.B. 1993). A large part of the biological nitrogen fixation in agricultural systems is derived from the cultivation of legumes. It is estimated that each year the soil is enriched with nitrogen from 100 to 300 kg per hectare, at the end of the vegetative period and after the decomposition of the plant. (Schlegel, 1992; Tate, 1994)

The interest in biological nitrogen fixation is current in organic agriculture. Fixed nitrogen is incorporated in the protein and increases not only yield, but also the quality of the yield. Significant influence for increasing legumes yields are seed materials treatment with active nitrogen fixing bacteria strains before sowing. Bacteria of lupine Bradyrhizobium sp. (Lupinus), differ in symbiotic properties (competitiveness and nitrogen-fixing activity), to chemotaxis with respect to the organic substances revealed in root exudates of lupine (amino acids, sugars, organic acids and their salts). (Kirichenko E., 2005)

In agricultural practice nitrogen fixing bacterial treatment is economically profitable. The effectiveness of nitrogen fixing bacteria is expressed as an increase in the plant fresh mass and the total nitrogen amount in the dry matter.

Lupine is one of the most popular legumes in the world. It is used as forage, green manure and in the last decade it has become more and more popular as a protein source. Lupine has almost the same protein content as soya, but contains more carotene and cellulose, low levels of starch and high levels of polysaccharides and raffinose. However while soy is widely used in human nutrition, lupine is mainly used as an animal feed. The main shortcoming of lupine as a food material is considered to be its alkaloid content, which means that it must be subjected to processing to remove alkaloids before it can be used as a food material.(Korol, 2003; Adomas B and Piotrowicz-Cieslak A, 2004)

The aim of this investigation was to test the symbiotic fixation effectivity of the bacterial strains on different lupine species during their ontogenesis. The tasks were to determine plant length, fresh weight, dry matter per plant, % of dry matter and the total nitrogen amount in the dry matter.

Materials and Methods

The experiments were carried out at the greenhouse of Latvia University of Agriculture, Faculty of Agriculture, Institute Soil and Plant Sciences. Three lupines species *L. luteus* variety 'Juno', *L. angustifolius* variety 'Sonet' and *L. albus* variety 'Wat' were used in the vegetation pot experiment. The experiments were placed in five replicas in 5 L Mitcherlich type pots with washed river sand, and fertilized with Kemira Grow How NPK 0-5-20 with microelements. Nitrogen was added as ammonium nitrate, 0.024 g per 1 kg of sand for inoculated plants and ten times more (0.24 g kg^{-1}) for the control variant, were plants were not inoculated. In each pot 5 seeds were sowed. Lupine seeds were inoculates with a commercial preparation, *nitragin*, marketed by the company 'Bioefekts' (www.bioefekts.lv) and with the strain *Rhizobium lupini* from the collection of the Institute of Soil and Plant Sciences.

All vegetation pots were placed in a greenhouse with a day temperature of 20 – 25 °C and a night temperature over 12 °C. The experiment lasted from August 18 till December 26 when plants were in the phase of the end of flowering and form pods. From October plants were illuminated with artificial light at morning and evening to obtain a total photoperiod 14 hours.

Plant length, fresh mass, dry matter, % of dry matter, and total nitrogen amount in the dry matter were measured. Plants for analysis were taken at intervals: juvenile plants (September 7); bud formation (November 3) and at the end of flowering, pod formation (December 26). The content of total nitrogen was determined with the Kjeldahl method at Latvia University of Agriculture Analytical Laboratory for Agronomy Research. (ISO -5983-2 : 2005)

Results and Discussion

The activity of *Rhizobium lupini* strains was characterized by yield and accumulated nitrogen in the plants. Plant seed treatment with bacteria stimulated lupine germination and the plant growth at the early stages of development. At the first interval (till September 7) all species showed a significant influence from the seed inoculation (Fig. 1.). This tendency remained till the second interval (till November 3). The best results were obtained with the institute bacterial strain. At the end of flowering period the species *L. luteus* had larger length in its untreated variant, the smallest length were observed in the variant with the institute collection strain treatment. Species *L. angustifolius* had largest length in the variant inoculated with 'Bioefekts' nitragin and the smallest length was observed in the untreated variant. In the species *L. albus* the major length was in the

variant inoculated with the collection strain and the smallest length was observed in the untreated variant. The main length increase of lupines was obtained at the intensive grown phase from November 3 till December 26. In the end of the flowering period the best results were from the species *L. luteus* variety 'Juno' The smallest length was observed in the species *L. angustifolius* variety 'Sonet', from the species *L. luteus* the length decrease was 34 %. Data processing showed that the plant length had a significant correlation to the lupine species and bacteria strain.

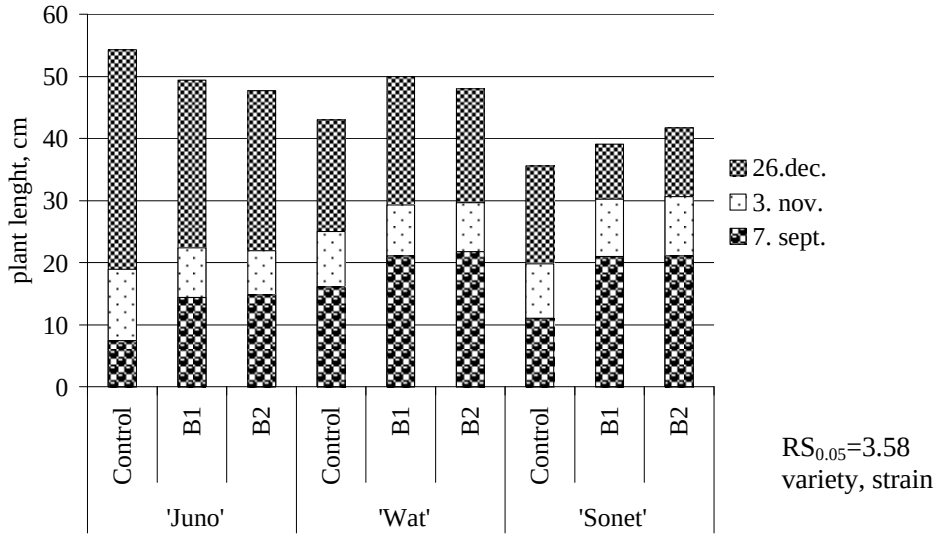

Figure 1. Plant length depending on the species and the treatment of the nitrogen fixing bacteria strain (Control - untreated variant; B1 – treated with company 'Bioefekts' nitragin; B2 – treated with collection strain).

For the lupine fresh weight gain observed at the end of the flowering period the best results were obtained for the species *L. albus* variety 'Wat'. Species *L. angustifolius* had the smallest fresh weight gain (Fig.2.). The effect of seed inoculation was observed till flowering. Plant fresh weight increased by 20.9 %. Lupine treated with the Institute strain weighed 8 % more than the "Bioefekts" one. At the end of the experiment data analysis showed a significant interconnection for the varieties 'Wat'and 'Sonet': lupines in the control variant (receiving higher nitrogen dosage) showed the lowest length, but higher fresh weight. Inoculated plants had longer length, but smaller fresh weight.

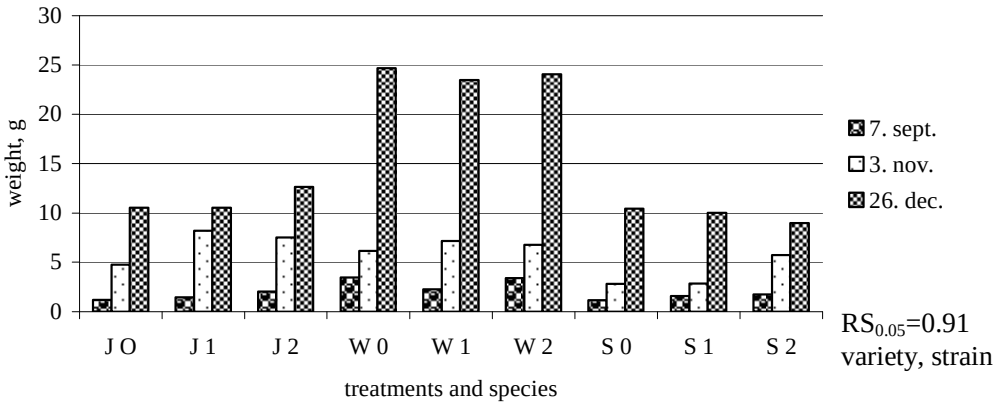

Figure 2. Plant weight depending on the species and the treatment of the nitrogen fixing bacteria strain (J- 'Juno', W-'Wat', S-'Sonet' – varieties, 0- untreated variant, 1- treated with nitragin and 2- treated with Institute collection strain)

The dry matter content was inspected in two intervals (September 7 and November 3). All the species after the first interval (September 7) showed a significant influence from the inoculation with lupine bacterial strains, with the exception of the species *L. luteus* variety 'Juno'(Fig. 3). The average increase of dry matter by 27 % was observed as the effect of inoculation. This regularity remained during all the periods of vegetation. At the end of the flowering period it was found that the species *L. luteus* had increased its dry matter content in the variants inoculated with the bacterial strains, the lowest was found in the control variant. Species *L. angustifolius* showed increased dry matter content in the variant inoculated with nitragin, the control variant had the lowest values. For the species *L. albus* the increased dry matter content was found in the variant inoculated with the collection strains, the lowest values were in the control variant. For the species *L. luteus* and *L. albus* increasing dry matter content was obtained in the variants inoculated with the collection strains. Processing the acquired data shows a significant correlation between lupine species and bacterial strains on plant dry matter.

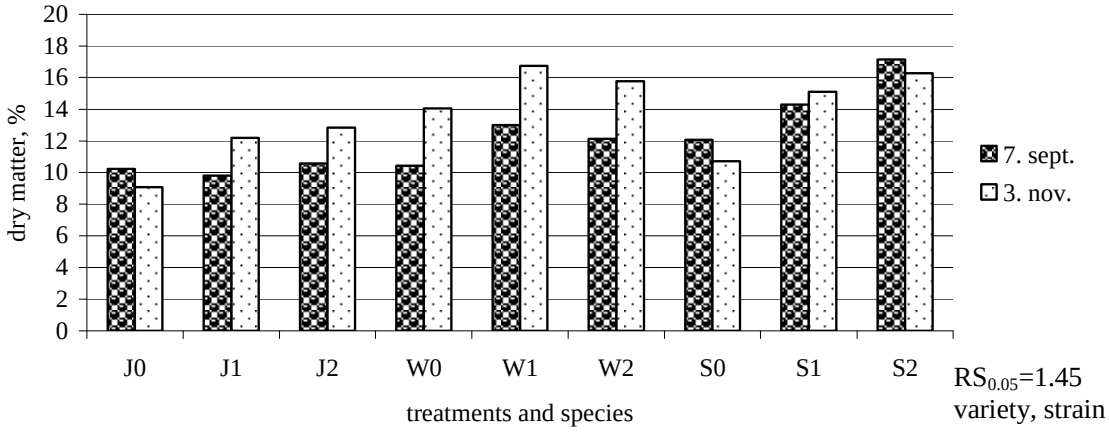

Figure 3. The plant weight depending on the species and the treatment of the nitrogen fixing bacteria strain (J- 'Juno', W-'Wat', S-'Sonet' – varieties, 0- untreated variant, 1- treated with nitragin and 2- treated with Institute collection strain)

The experiments showed the effect of inoculation on the protein content of lupine. Till the flowering phase there was no significant effect of the inoculation on the protein content, but further protein accumulation was influenced by the nitrogen supply. The formed symbiotic system did not assure a sufficient nitrogen supply to the plants.

The total protein amounts in dry matter were higher in the control variants (10 times higher nitrogen dosages). Comparing the total amounts of protein in the lupines inoculated with nitrogen and the collection strains gave the following results: For for species *L. luteus* variety 'Juno' the best symbiotic system was formed with the collection strains. The total nitrogen content increased in the dry matter by 6% in comparison with the nitrigin treatment. For the species *L. angustifolius* variety 'Wat' the best symbiotic system was formed with the collection strains, giving a increase of total nitrogen in the dry matter by 5 %. For the species *L. albus* variety 'Sonet' the best symbiotic system was formed with nitragin increasing the total nitogen in the dry matter by 10 % compared to the collection strain.

Conclusions

The plant length depends on plant species. The smallest length was observed in the species *L. angustifolius* variety 'Sonet', the largest are the plants of the species *L. luteus* variety 'Juno'.

The inoculation of plant seeds promotes an increase in the plant length untill the phase of bud formation. There are no significant differences between *Rhizobium lupini* strains.

The largest fresh and dry weight was observed for the *L. albus* variety 'Wat', but species *L. angustifolius* had the smallest fresh and dry weight.

The largest dry matter content in plants untill the stage of bud formation was observed for inoculated plants.

The effectiveness of *Rhizobium lupini* strains are species dependent.

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VĀRPU FUZARIOZES IZPLATĪBA UN MIKOTOKSĪNU RISKS ZIEMAS KVIEŠU SĒJUMOS LATVIJĀ
OCCURRENCE OF THE *FUSARIUM* SPECIES AND THE RISK OF MYCOTOXINSASSOCIATED WITH HEAD BLIGHT IN WINTER WHEAT IN LATVIA

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Abstract

During 2005-2006 the monitoring study was conducted by the Latvian Plant Protection Research Centre. The incidence of Fusarial head blight (FHB) in winter wheat was investigated in various Latvian regions. The distribution of the *Fusarium* species, isolated from samples representing production fields and variety testing trials with several winter wheat varieties and identified by V.Bilai (1977) methods is described. The incidence of FHB varied from 2 to 35% in 2005, from 1.66 to 25.2% in 2006 and there were no differences between regions. In 2005 - 5, in 2006 - 6 the *Fusarium* species the caused FHB were identified. The most frequent were: *F. poae, F. culmorum, F. gibbosum*, besides *F. avenaceum* var. *herbarum, F. sporotrichoides, F. moniliforme* were found. *C*ontamination of grain with mycotoxins was examined – in 2005 for DON, in 2006 – for DON, ZEN, T-2. Of 7 samples only 1 in 2005 and of 70 samples only 3 in 2006 were contaminated with DON. The contamination level was very low – 24, 36, 36 and 28 µg kg^{-1}, which is below the level fixed in the EC Regulation 466/2001 – 1250 µg kg^{-1}.

Key words

Fusarium species, incidence, frequency, mycotoxins, winter wheat