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IMPACT OF INOCULANT AND FOLIAR FERTILIZATION ON ROOT SYSTEM PARAMETERS OF PEA (*PISUM SATIVUM* L.)

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Abstract. In recent years, sustainable crop development has played a key role in current strategies to improve roots activity, which increase nutrients uptake in pulse crop. Our study presents the relationship between root system morphology, inoculant application with and without foliar fertilization and nitrogen accumulation in soil and plants. Two inoculants: Nitragina and IUNG, foliar fertilizer (Photrel), as well as two pea cultivars were studied in three years (2009–2011) period. The research has shown that bacterial inoculants have significant influence on the selected parameters of pea root systems. Gel inoculant significantly increased mean root diameter (0.44 mm), compared to control (0.33 mm), whereas combination of Nitragina inoculant with micronutrient fertilization significantly increased root length density (1.05 $cm \cdot cm^{-3}$), compared to control (0.85 $cm \cdot cm^{-3}$). Additionally, the bacterial inoculant IUNG has significantly decreased the root length density in roots classes between 0.2–0.5 mm in the most humid year. The impact of inoculants on roots parameters was strongly related to weather conditions. In a dry year, a significant decrease of mean root diameter, specific root length and increase of root dry mass were observed. Nitrogen accumulation in seeds significantly increased after gel inoculant application. A higher N content was proven in the fodder cultivar, but the edible cultivar was observed to accumulate more N in the seeds, which caused a Nitrogen Harvest index for this plant (80.0%).

Keywords: N-content, N-accumulation, root features, nitrogen index

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INTRODUCTION

Biotechnological practices such as the application of bacterial inoculants have been tested throughout the world for many years (Gupta *et al.* 2015). A positive aspect of the introduction of this technology is the attempt to restrict the use of nitrogen fertilizers, which means the improvement in soil quality according to sustainable development approach (Barea 2015, Hassen *et al.* 2014, Tena *et al.* 2016). Presently, soil microorganisms play essential roles in agriculture. Inoculating the soil with *Rhizobium* influences the increase in nodulation, nitrogen absorption by the plants, and increases the legume yield (Tena *et al.* 2016). However, bacterial inoculants not always prove effective; it depends on the quality of the inoculant and on the specific environmental factors. The plants reaction to inoculation is determined by various factors such as the presence of a natural population of *Rhizobium* bacteria, N availability, physical and chemical limitations of the soil, weather conditions (Uyanöz and Karaca 2011). Natural *Rhizobium* strains in the soil are in some way limiting to the effectiveness of bacterial inoculants, which is why the right selection of strains and compatibility with the soil can be a sort of blueprint for the right way to increase yield (Vacheron *et al*. 2013, Yadav *et al*. 2011). Even though the bacterial inoculants are being perfected by selection of better *Rhizobium* strains to increase the effectiveness of nitrogen absorption and resistance to environmental stress, bacterial inoculants do not always influence the plants growth or yield (Tena *et al*. 2016). Zając *et al*. (2013) established that application of seed inoculation with *Rhizobium* did not affect the GAI values and seed yield. However, the previous studies by Przemeck and Schrader (1981) proved that there exists the relationship between nitrogen assimilation capacity of the root and the mineral nutrition of the plant, which is linked by carbohydrate synthesis in the leaves and the supply to the root of assimilates. Zhang *et al.* (2015) have proven a large genotype variation in pea root biomass production and the root architecture in laboratory conditions. The authors noticed a link between the roots effectiveness of absorption of mineral nutrients from the soil and seed productivity.

Examining the synergistic links between the genotype variation of pea with the kind of inoculant, it can be assumed that the plants accumulate the nitrogen contained in the soil more effectively. Some researchers indicate that rhizosphere bacteria are more significant to nitrogen accumulation than pea morphology. Rhizosphere bacteria influence phytohormons, causing significant changes in root system morphology: the number of roots and their length (Vacheron *et al.* 2013). The morphological variation in *Fabaceae*, caused by modifications of the root system, can cause differences in nutrient absorption from the soil (Desbrosses and Stougaard 2011). Changes in plant metabolism can affect the mutual relations between plants and bacteria strains, which results in the changes of the root system and the number of root nodules. Since the morphological variation of pea varieties is large, no detailed research pertaining to the influence of bacterial inoculants on root morphology or the effectiveness of their N absorption has been performed yet.

Works on the cultivars and environment interaction (*Rhizobium* strains) on nodulation effectiveness, aboveground biomass growth and nitrogen accumulation were described in a limited scope (Podleśny *et al*. 2014, Zhang *et al.* 2015). Podleśny *et al*. (2014) have shown that *Rhizobia* influence the development of legume roots and nodule induction. However, their effect on the root structure is less known, therefore, in our research we have tried to prove that root morphology is shaped by *Rhizobium* strains inoculation. What is more, we have tried to prove that a better developed root system is more effective in water and nutrient absorption, which causes better accumulation of N in the seeds. Another aspect of our research was to prove that foliar fertilization has a positive influence on the shaping of pea morphological characteristics. Until now, no literature has contained information on the influence of foliar fertilization on the morphological characteristics of root systems in legumes.

The aim of the research was to test two hypotheses in field conditions: i) the root system is modified by the use of bacterial inoculants and foliar fertilizer; ii) pea cultivars have different opportunity for nitrogen accumulation in soil and above-ground parts of the plant, which is related with roots activity.

MATERIALS AND METHODS

Experimental designs and agronomic management

A field experiment was conducted at the Experimental Plant Cultivation Centre in Modzurów (50°09'N 18°07'E). The soil of the experimental field was Haplic Phaeozem (WRB 2015). The soil pH was 6.3 (pH in 1 mol·dm^{-3} KCl) and the nutrients of the topsoil layer were (per 100 g soil): phosphorus – 19.1 mg; potassium – 21.7 mg, and magnesium – 10.1 mg. The total mineral nitrogen content in the soil at a 0–90 cm depth, was about 73.6–77.3 kg·ha^{-1}, and the C/N ratio was in the range of 9.0–9.5. The amount of *Rhizobium leguminosarum* bv. *viciae* in soil (1 g) was moderate ($1.7–7.1 \times 10^2$) (Martyniuk *et al.* 2005).

The experimental design was split-split plot in a randomized complete block design with three replications. The plot size was 9 m^2. The precrop for pea (*Pisum sativum* L.) in each year (2009–2011) was winter wheat. Pre-sowing fertilization (kg·ha^{-1}) amounted to Nitrogen – 20, phosphorus (P_2O_5) – 48 and potassium (K_2O) – 72, in the form of Polifoska (8-24-24). Each year, the following amounts of seeds were sown per square meter: 120 afila, ‘Tarchalska’ cultivar, and 100 of standard-leaved ‘Klif’. Assumption of different amount of seeds density was related with different efficiency of germination process between species. After plant germination in seedling stage, the amount of plants per square meter was equal.

One day before the sowing, the seeds of each tested pea cultivars were coated with Funaben T™ 75 DS/WS and inoculated: granular formula – Nitragina™ by BIOFOOD® and IUNG gel. Both inoculants contained *Rhizobium leguminosarum* bv. *vicae*. The Nitragina contained 11 CFU g^{-1}, while IUNG gel – 13 CFU g^{-1}. *Rhizobia* isolation was performed using the standard procedure described by Vincent (1970). The pea seeds were sown at a depth of 6 cm. At the beginning of the budding stage (BBCH 51), the microelement fertilizer Photrel™ (150 g of B, 210 g of Mn, 12 g of Mo, 400 g of MgO, and 1,081 g of SO_3), was applied to leaves in the amount of 3 $dm^3 \cdot ha^{-1.}$

Plant, root and soil analysis

In the flowering stage, 15 plants from each plot were collected and fresh mass was assessed. The samples were dried at the temperature of 70°C for 48 h. Dry plants were weighted and nitrogen content was analyzed. In the maturity stage, 15 plants from each plot were collected and biometrical analysis was used. The plants were separated into number of pods, number of seeds, petioles, leaves and stems. Based on dry biomass and seeds, harvest index was calculated. In the dried parts of the pea plants, the total nitrogen content was determined by the Kjeldahl method. N accumulation was calculated as N content × dry parts of plants. Based on those data, the Nitrogen Harvest Index (NHI) was calculated according to the formula presented by Neugschwandtner and Kaul (2015): NHI = N seed/N total ($mg \cdot g^{-1}$ 100).

In the flowering stage (BBCH 60–69), soil samples of each plot were taken in the topsoil (0–30 cm). Soil samples together with pea roots were taken with a root sampler produced by Eijkelkamp®. The diameter of the soil cylinder cut out for the samples was 7 cm, and its height was 15 cm. Each sample was rinsed with an automatic, hydraulic-pneumatic root flusher. The cleaned roots were transferred onto filter paper to remove the surplus of water. Fresh roots were placed on the surface of a scanner (Epson Perfection 4870 Photo®) and scanned in 1,200 dpi resolution. All images obtained were saved in the .tif format. The roots were dried for 4 h at the temperature of 70°C to determine the dry matter content. The images of roots were analyzed with APHELION v 3.2 software, designed specifically for image analysis. On the basis of the results achieved, the following factors were calculated: Root length density (RLD) = L/V, where L is root length in cm, and V is the volume of the sample in cm^3; Mean root diameter (MRD) calculated as the weighted arithmetic mean of root diameter; Specific root length (SRL) = L/M, where L is root length in cm, and M is a mass in g; Root dry mass (RDM) in g.

In addition, during the flowering stage, three soil samples were collected for chemical analysis from each plot. The soil was sampled from three layers of the soil profile: 0–30; 30–60, and 60–90 cm. The soil samples were air dried and milled for chemical analysis. In the soil samples, the following properties were determined: the content of total carbon (TC) and nitrogen (TN), using a LECO CNS 2000 automatic analyser [LECO 1996]. NO_3^- and NH_4^+ content was determined by spectrophotometric methods using a Merc Spectroquant Pharo 100 spectrophotometer.

Statistical analysis

The results were statistically analyzed by the variance analysis method using the Statistica 10.0 software (Stat Soft, Inc., USA). Significant differences (HSD) for the traits were verified using Tukeys' test at the significant level of $p<0.05$ and $p<0.01$.

Meteorological conditions

During the years of pea vegetation, the weather conditions were varied (Table 1). The year 2009 was outstanding compared to other years of study. The first period of plant vegetation (April and May) was dry owing to low precipitation (5.6 mm and 55 mm, respectively) and high temperature (12.5ºC, 14.2ºC, respectively). However, the subsequent months of vegetation – June (109 mm) and July (137 mm) – were extraordinarily humid for pea vegetation. The year 2010 has proven the most humid of all in the three-year period of the experiment, mostly because of abundant precipitation in May (193 mm) and July (208 mm). In 2011, the amounts of precipitation were lower when calculated for the whole growing season, but July of 2011 was quite humid with the monthly precipitation amount of 167.5 mm. Air temperature was similar during 2010–2011. The highest temperature was noted at the end of the maturity stage (July), while the lowest temperature was noted during the sowing (the end of March).

Table 1. Weather conditions

Parameters	Years	March	April	May	June	July	August
Temperature (ºC)	2009	3.8	12.5	14.2	15.9	20.0	19.2
	2010	4.0	7.5	11.7	16.7	20.4	18.5
	2011	2.0	9.7	13.2	17.4	17.3	18.9
Rainfall (mm)	2009	96.6	5.6	55.2	109.2	137.0	55.4
	2010	17.0	66.5	193.2	103.5	208.5	95.1
	2011	31.1	29.2	71.5	99.5	167.5	73.2

RESULTS

Root architectures

RDM was not affected by the cultivar, inoculate or the foliar fertilizer (Table 2). However, we gained a significant ($p<0.01$) interaction between cultivar and inoculate application (Fig. 1a). Inoculates application (IUNG, Nitragina) substantially decreased the RDM comparing to control conditions in fodder cv. Klif, while inoculates considerably increased RDM comparing to control in edible cv. Tarchalska. In control conditions, a significantly ($p<0.01$) higher RDM was obtained in cv. Klif, in relation to cv. Tarchalska. RDM was affected by weather conditions. In dry 2011 year, there was established significantly higher RDM, compared to humid, 2009 year (Table 2). Significant ($p<0.05$) interaction between the foliar fertilizer application and the year was showed (Fig. 1b). A significant ($p<0.05$) increase ($1.4\ \mathrm{mg \cdot cm^{-3}}$) in RDM was noticed in dry year 2011, when Photrel was applied, in relation to 2009 ($0.65\ \mathrm{mg \cdot cm^{-3}}$) and 2010 ($0.85\ \mathrm{mg \cdot cm^{-3}}$).

RLD was not affected by the inoculate or the foliar fertilizer (Table 2), however a significant ($p<0.05$) interaction between inoculate and foliar fertilizer was noticed (Fig. 2a). Application of powder inoculate (Nitragina) with the foliar fertilizer (Photrel) significantly increased RLD (1.18), while opposite interaction (0.675) was observed after IUNG application (Fig. 2a). Significant interaction between the inoculate and the year proved that water shortage during plant vegetation (2011) significantly decreased the RLD value (Fig. 3a). In dry year, IUNG inoculant significantly increased in RLD ($1.05\ \mathrm{cm \cdot cm^{-3}}$), compared to Nitragina. An opposite phenomenon was noticed in humid year (2010), where IUNG inoculant was least effective.

Additionally, significant ($p<0.05$) interaction was observed between cultivars and study years (Fig. 3b). Fodder cultivar Klif obtained higher RLD value ($1.05\ \mathrm{cm \cdot cm^{-3}}$) in 2009, compared to 2010. Particular analysis of RLD root class confirmed significant ($p<0.05$) differences (Fig. 4a, b). In the comparison of RLD among the cultivars, differences were observed only in 2009. The fodder cultivar Klif has created significantly more roots in classes between 0.2 and 0.5 mm (Fig. 4a). Compared inoculants had slightly influenced RLD variation in the root density classes, which were examined during a three-year study (Fig. 4b). According to Figure 4b, in 2010, there were marginal differences between treatments regarding root classes 0.1–0.2 mm. However, in class 0.2–0.5 mm, IUNG treatment showed significantly ($p<0.05$) lower RLD than control and Nitragina. 2010 was the only year when a significant density of root classes (0.1–0.5 mm) was observed in conditions.

Bacterial inoculants had no significant influence on MRD, because the inoculants application caused a slight decrease in MRD (Table 2). Significant

Table 2. Comparison of root indices depending on cultivar, pre-sowing seed inoculate with bacteria and foliar fertilizer (means of three years)

Treatments		Root indices			
		RDM ($g\cdot cm^{-3}$)	RLD ($cm\cdot cm^{-3}$)	MRD (mm)	SRL ($cm\cdot g^{-1}$)
Cultivar (C)	Klif	0.001024	0.899	0.451	1002.1
	Tarchalska	0.000969	0.802	0.443	1200.1
HSD $_{0.05}$		ns	ns	ns	ns
Inoculant (I)	control	0.00103	0.923	0.462	1446.5
	Nitragina	0.000972	0.837	0.439	929.2
	IUNG	0.000988	0.792	0.441	927.5
HSD $_{0.05}$		ns	ns	ns	ns
Foliar fertilizer (F)	without	0.001004	0.871	0.447	969.2
	Photrel	0.000989	0.831	0.448	1232.9
HSD $_{0.05}$		ns	ns	ns	ns
Year (Y)	2009	0.000745	0.918	0.532	1733.3
	2010	0.000891	0.845	0.422	957.6
	2011	0.001354	0.789	0.389	612.4
HSD $_{0.05}$		0.000156	ns	0.035	879.9
C × I		**	ns	ns	ns
C × F		ns	ns	ns	ns
I × F		ns	*	*	ns
C × Y		ns	*	ns	ns
I × Y		ns	*	**	ns
F × Y		*	ns	ns	ns

ns – not significant, * significant difference $p<0.05$, ** significant difference $p<0.01$, ± – standard error

($p<0.05$) interaction between the bacterial inoculate and the foliar fertilizer was shown only for 2011 (Fig. 2b). A significant ($p<0.05$) increase (0.44 mm) in MRD was noticed when bacterial inoculate (IUNG) was applied (Fig. 2b), in relation to control (0.33 mm). The foliar fertilization was most effective in increasing MRD without inoculate application.

Weather conditions had relevant impact on the MRD value. Significantly higher MRD was noticed in 2009, compared to 2011. Additionally, there was found a significant ($p<0.05$) interaction between the inoculant type and years (Fig. 5). A significant ($p<0.05$) decrease in MRD (1.05 $cm\cdot cm^{-3}$) was noticed in 2011, in relation to 2009 (0.8 $cm\cdot cm^{-3}$) when inoculant (Nitragina) was applied.

The SRL value was not significantly changed by the bacterial inoculant or by the foliar fertilization (Table 2). The only tendency noted was the one of SRL decrease after inoculant application. This fact may be related to the decrease in environmental stress because of the increase in mineral nitrogen content in the

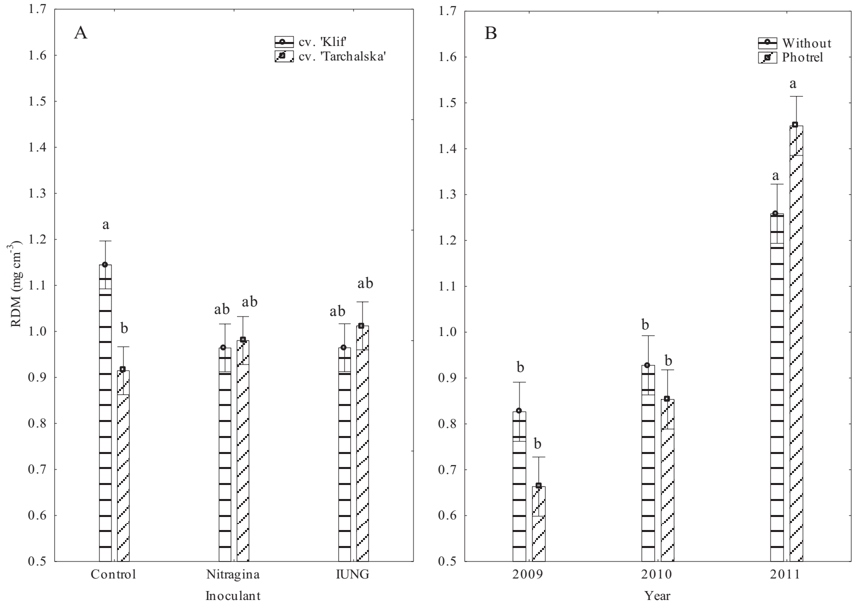

Fig. 1. Root dry mass (RDM) distribution depending on (A) cultivar and inoculate type, (B) foliar fertilization application and years

Different letters on bars indicate significant differences of mean values. Bars represent standard errors.

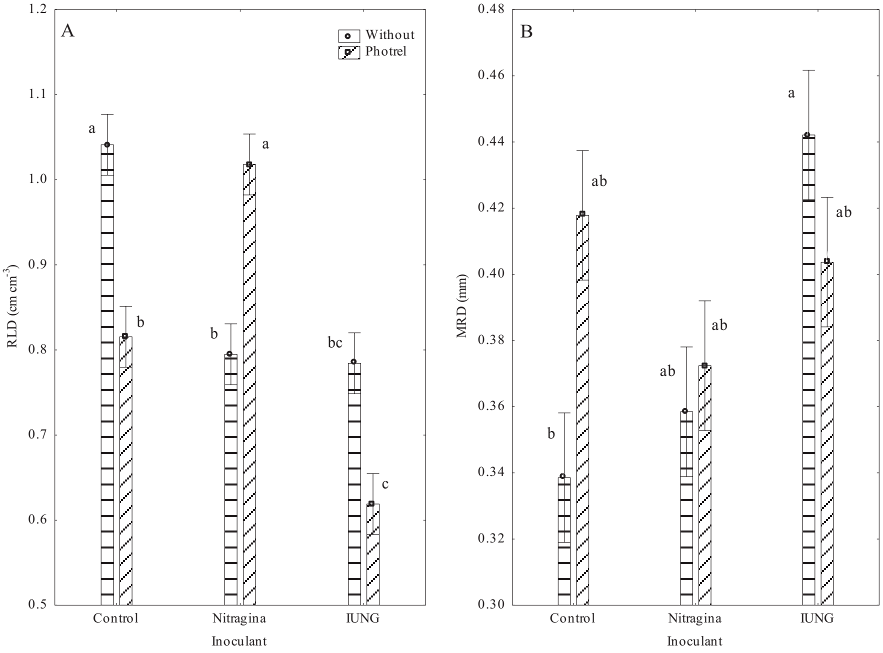

Fig. 2. Distribution of root length density (RLD) (A) and mean root diameter (MRD) (B) depending on inoculate type and fertilizer application

Different letters on bars indicate significant differences of mean values. Bars represent standard errors.

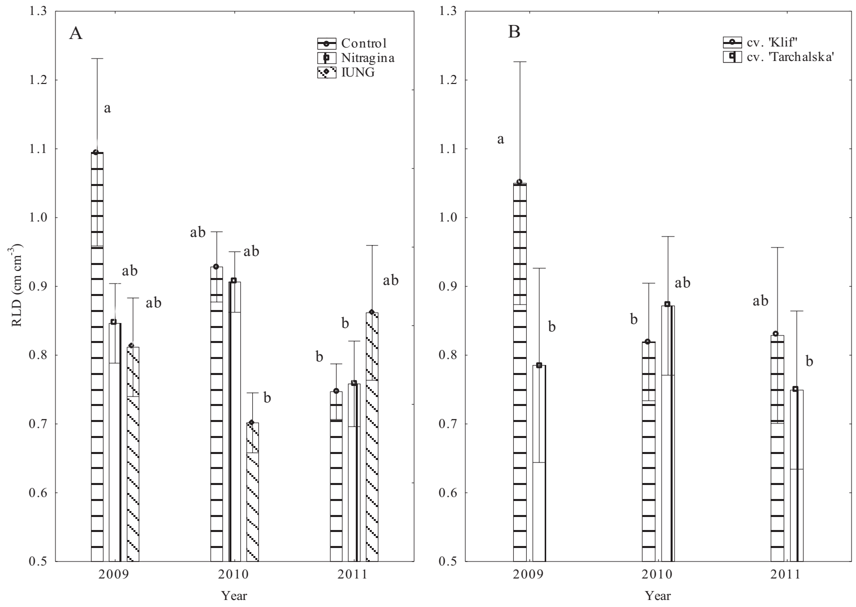

Fig. 3. Distribution of root length density (RLD) depending on (A) inoculate type and year (B) cultivar type and year

Different letters on bars indicate significant differences of mean values. Bars represent standard errors.

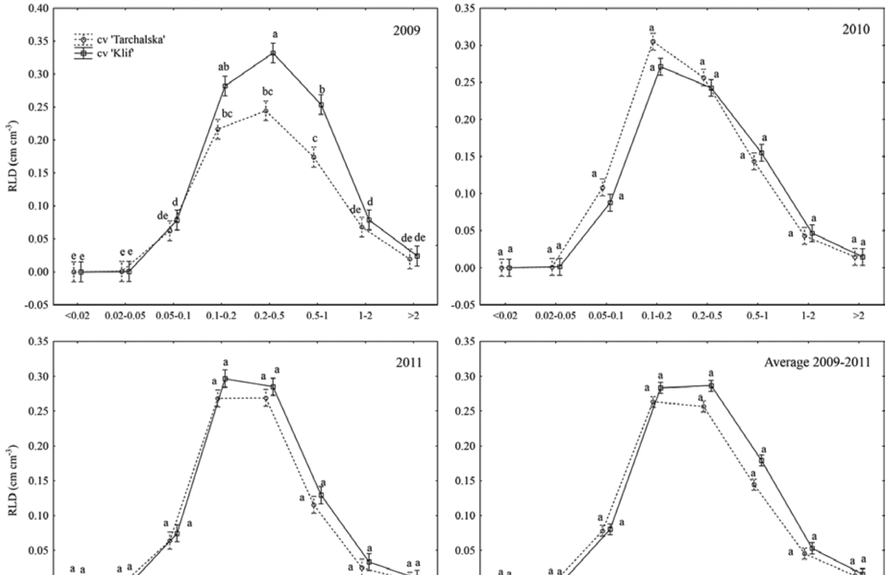

Fig. 4a. Root length density (RLD) distribution of pea cultivars depending on years of vegetation

Different letters on bars indicate significant differences of mean values. Bars represent standard errors.

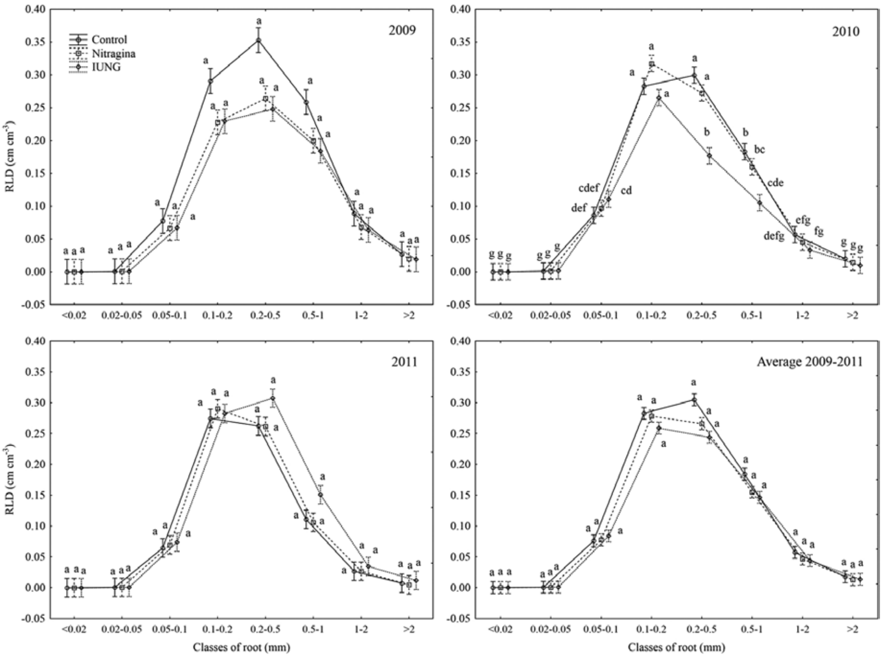

Fig. 4b. Root length density (RLD) distribution of inoculate type depending on years of vegetation

Different letters on bars indicate significant differences of mean values. Bars represent standard errors.

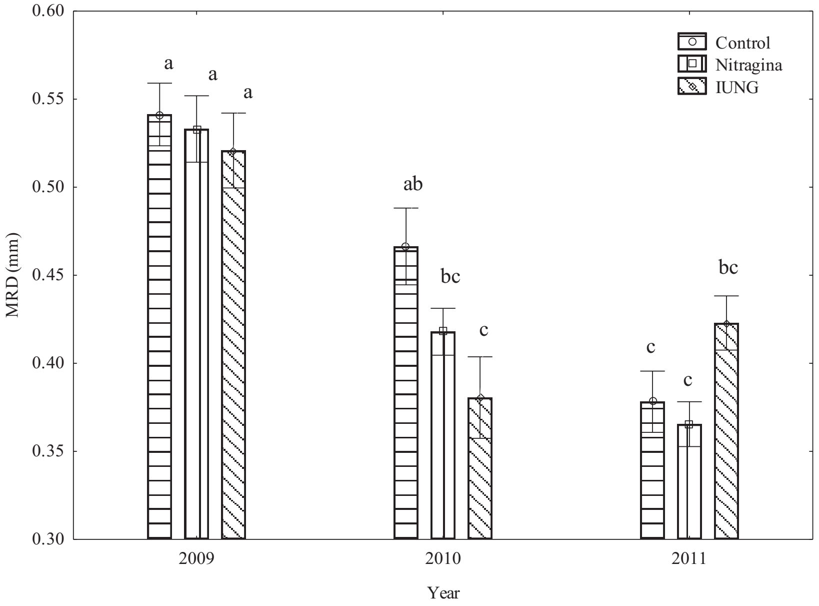

Fig. 5. Distribution of mean root diameter (MRD) depending on the inoculate type and year

Different letters on bars indicate significant differences of mean values. Bars represent standard errors.

soil. A large decrease in SRL was shown after bacterial inoculants were applied, in comparison with control conditions, which proves greater root system activity searching for nutrients, which means greater nitrogen content in the soil. The presence of foliar fertilizer had no significant influence on the shaping of SRL, but it was observed that SRL considerably increased after the foliar fertilizer was applied. SRL was affected only by weather conditions. A significant increase in SRL was noticed in 2009, which indicated less favourable soil conditions for roots development.

Allocation of nitrogen in the plant and soil – root activity

The nitrogen content, as well as the accumulation of fresh and dry matter in the pea plants, were notably varied at the flowering stage (Table 3). The foliar fertilizer significantly ($p<0.05$) increased the amount of the accumulated fresh mass of pea shoots in the cv. Klif, characterized by regular foliage. The nitrogen content in the dry mass of pea plants depended on agrobiological properties of the cultivars, which is why the cultivar with regular foliage (Klif) had a higher content of this element. Due to the higher shoot mass and nitrogen content, there was considerably more nitrogen in the pea plants with regular foliage. The IUNG bacterial vaccine allowed for the accumulation of larger amounts of nitrogen in plant shoots, probably on account of the considerably higher mass. At the full flowering stage of pea, in the above-ground biomass, with regular foliage, accumulated more nitrogen than the afila pea morphotype (cv. Tarchalska). The foliar application of microelement fertilizer shows significant ($p<0.05$) changes in the amount of nitrogen deposited in the above-ground mass of pea. Higher N accumulation was observed after foliar fertilizer's application in the Klif cultivar. Additionally, it was noted that the increased MRD and RLD in the Klif cultivar significantly ($p<0.05$) conditioned the increase in N amounts in the plant and in the soil. The edible cultivar (Tarchalska), characterized by higher SRL, has accumulated more N in its seeds.

Table 3. Nitrogen content and accumulation of fresh and dry mass of single pea plants and on surface unit – flowering stage (means of three years)

Items	Cultivar		HSD	Inoculant			HSD	Foliar fertilizer		HSD
	Tarchalska	Klif	0.05	control	Nitragina	IUNG	0.05	without	Photrel	0.05
FM ($\mathrm{g \cdot plant^{-1}}$)	34.5	44.2	8.66	36.9	37.5	43.6	ns	37.3	41.3	2.87
DM ($\mathrm{g \cdot plant^{-1}}$)	5.56	6.87	ns	5.85	5.89	6.90	ns	6.02	6.4	ns
DM ($\mathrm{g \cdot kg^{-1}}$)	159.0	154.8	ns	157.7	156.7	156.4	ns	159.4	154.5	ns
NC ($\mathrm{g \cdot kg^{-1}}$)	27.2	34.4	3.57	29.9	30.5	32.0	1.99	30.2	31.4	1.18

Items	Cultivar		HSD	Inoculant			HSD	Foliar fertilizer		HSD
	Tarchalska	Klif	0.05	control	Nitragina	IUNG	0.05	without	Photrel	0.05
NA (mg·plant^{-1})	153.1	237.2	30.05	176.8	183.7	225.0	48.60	186.0	204.4	17.9
TC/TN in soil	7.96	8.42	ns	7.90	8.27	8.40	ns	8.08	8.30	ns
NS (kg·ha^{-1})	121.6	108.2	0.59	116.1	113.6	115.0	0.763	119.9	110	0.54

FM – fresh mass, DM – dry mass, NC – nitrogen content in plant, NA – nitrogen accumulation, TC/TN – total carbon/total nitrogen ratio, NS – nitrogen content in soil, ns – not significant

Impact on TC/TN ratio

The narrower the total TC/TN ratio, the more nitrogen was used by the plants (Table 3). A tendency for an increase in the total TC/TN ratio in the deciduous cultivar after the application of the IUNG inoculant and the Photrel microelement was observed. The lower amount of nitrogen in soil resulted from higher accumulation of nitrogen in a plant. Low total TC/TN values because of not using bacterial vaccine as well as the lack of microelements absorbed may indicate a slowdown in the organic matter decomposition in the soil. Significant ($p<0.05$) differences in the amount of accumulated nitrogen in soil were found. An increase in nitrogen in the soil was obtained in treatments of the deciduous cultivar, in control conditions – without using the inoculant or the microelement fertilizer. Lower nitrogen accumulation in plants in control treatments was confirmed by its higher amount in the soil.

Nitrogen content and accumulation in the above-ground parts

The nitrogen content and accumulation in different parts of plant was related to the type of pea cultivars (Table 4). Klif cultivar contains significantly higher nitrogen contents in above-ground parts of the plant such as stem, petiole, pods, seeds, compared to cv. Tarchalska. However, a slight increase of nitrogen accumulation in seeds of Tarchalska resulted from higher dry mass (DM) and consequently from higher nitrogen harvest index (Tables 3, 4). The edible cultivar (Tarchalska) reached a higher value of the nitrogen index (80.0%). The pea cultivar with regular foliage (Klif) accumulated only 68.6% of nitrogen in seed. N accumulation in seeds was slightly higher in the plants to which the IUNG inoculant was applied, compared to the control treatment. The foliar fertilizer in combination with inoculant did not increase nitrogen accumulation in seeds. The significant increase of nitrogen accumulation in seeds was observed only in treatment with IUNG application without the foliar fertilizer (Fig. 6d).

Table 4. Content and accumulation of nitrogen in parts of single plant of pea cultivars in full maturity stage as well as nitrogen index (means of three years)

Items	Cultivar	Mature part of shoot				Nitrogen index (%)
		stem	petiole	pods	seeds	
NC (g·kg^{-1})	Tarchalska	10.70	7.08	6.60	35.47	-
	Klif	14.90	10.98	9.38	37.91	-
	HSD	2.96	1.34	ns	ns	-
NA (mg·N plant^{-1})	Tarchalska	42.10	1.87	7.50	205.4	80.0
	Klif	68.33	3.30	11.18	180.8	68.6
	HSD	18.17	0.49	ns	ns	6.7

NC – nitrogen content, NA – nitrogen accumulation, ns – not significant

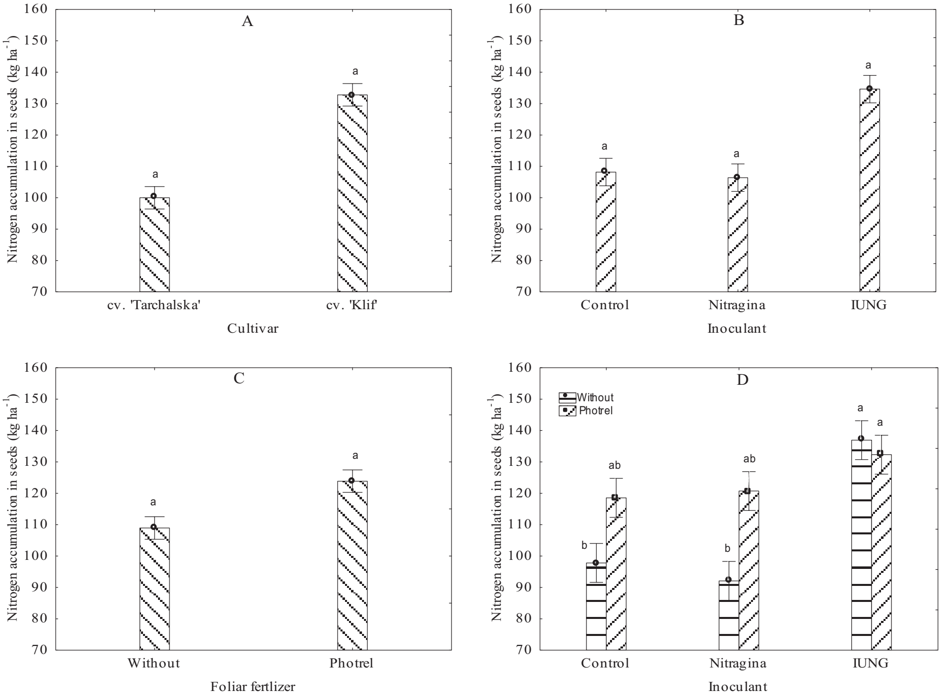

Fig. 6. Nitrogen accumulation in seeds depending on cultivars (A), vaccines (B), foliar fertlizer (C) intreraction vaccines and foliar fertilizer (D)

Different letters on bars indicate significant differences of mean values for nitrogen accumulation. Bars represent standard errors.

DISCUSSION

In field conditions, the application of the bacterial inoculants (IUNG, Nitragina) slightly decreased the RDM of the foliage cultivar, and slightly increased the RDM of the edible pea cultivar. In control conditions, a significantly higher

RDM was obtained in the foliage cultivar (Klif). This partly confirms the findings of Fageria and Moreira (2011), who claim that increasing nutrient supplies in the soil may decrease root length, but increase root weight. In laboratory conditions, Hassan *et al.* (2015) indicated that rhizobacterial isolates increased all measured physical, chemical and enzymatic growth parameters of wheat. *Rhizobacteria* increased root length, shoot length, dry weight, N uptake by shoots and by roots. The results obtained in our studies were consistent with those reported earlier by Hassan *et al.* (2015). The inoculant application significantly increased N accumulation in pea shoots. Additionally, we showed that foliar fertilization with micronutrients also has a significant impact on N contents in plant biomass. Mitova and Stancheva (2013), who compared different fertilization sources, indicated that foliar fertilization slightly increased N levels in plants. Ahemad and Kibret (2014) have reported that hormone production by plant-growth promoting rhizobacteria is helpful for better nutrient mobilization and the availability for plants, and could result in the development of much better germination of seeds and longer roots, which subsequently affects the growth of the host plant. In our studies, *Rhizobium* inoculant had a significant influence on the root length density only in 2010, which was characterized by higher levels of rainfall at the flowering stage. In 2010, significant RLD classes between 0.1–0.5 mm were observed in control conditions, while lower RLD classes were obtained in crops on which the IUNG inoculant was applied.

This confirms the findings of Ardakani *et al.* (2009), who claimed that increasing of soil water deficit significantly affected root dry weight, specific root mass and root length. Including *Rhizobium* inoculate associated with irrigation system significantly increased all root parameters in lucerne. According to Guleria *et al.* (2014), the application of selected rhizobial strains resulted in adverting the effect of moisture stress, these positive effects of bacteria on seed germination might be attributed to increased water use efficiency and stimulation of root growth. This fact is of crucial importance, as, according to Nie *et al.* (2015), bacteria influence total root length. Additionally, the authors claimed that bacteria strains increase root density, which increases the root absorption area. However, Rao *et al.* (2016) claim that roots structure is shaped by soil quality. Our study confirmed these findings only slightly, because a small decrease of specific root length was observed after the bacterial inoculants application. However, these results indicate that roots were able to efficiently penetrate the soil, and that had a relevant impact on nitrogen accumulation in the seeds. SRL was significantly related to weather conditions. In the dry year of study, the higher value of SRL was observed. Klimek-Kopyra *et al.* (2015) and Atkinson (2000), claimed that SRL reflects the potential for plants to exploit the soil, by altering their potential for nutrient and water uptake. Carranca *et al.* (2015) indicated that 7–11% of total N was associated with roots and nodules activity. Additionally, they noticed that allocation of 11–14 kg of N fixed t^{-1}

belowground dry mass in legume, represents half of the amount of aboveground plant. Fageria and Moreira (2011) have showed that among the genotypes of one species subjected to the research, genotypes with large root mass absorb nutrients more effectively. In our studies, the nitrogen content in the dry mass of pea plants depended on agrobiological properties of the cultivars. The cultivar with regular foliage (Klif) had a higher content of nitrogen in the soil and in biomass, which was related with MRD and RLD parameters. However, the afila cultivar (Tarchalska), characterized by larger root mass, has accumulated more (80.0%) nitrogen in its seeds. This confirms the findings of Fageria and Moreira (2011), who claimed that better root growth may be responsible for higher absorption of nutrients and water in treatments, which resulted in higher yield. Bai *et al*. (2015) claim that arbuscular mycorrhizal fungi (AMF) and *Rhizobium* symbiosis has great potential in enhancing productivity through better proliferation of the root system and improved soil fertility status. Additionally, inoculation of AMF and *Rhizobium* can save up to 25% fertilizer N in pea. The results obtained in our investigations are in excellent agreement with the previous study of Hayat *et al.* (2010), who came to the conclusion that growth promoting rhizobacteria increases the mobility and availability of plant nutrients in the soil, and, as a result, increases the nutrient uptake of the plants.

CONCLUSIONS

1. *Rhizobium* inoculant of legume crops is a very suitable approach to increase nitrogen accumulation both in the biomass and the soil, however, it is still dependent on a multifactor interaction.
   Nitrogen content and accumulation in the plant significantly depended on *Rhizobium* inoculation and cultivar type. A significant increase of nitrogen content in plants and significant decrease of nitrogen content in soil was observed after gel inoculant application. The edible pea cultivar accumulated more nitrogen in seeds, and gained higher nitrogen index (NI = 80.0%).
2. Bacterial inoculants had a significant influence on root parameters. Gel inoculant significantly increased (0.44 mm) MRD, compared to control (0.33 mm). Combination of Nitragina inoculant with micronutrient fertilization significantly increased RLD (1.05 cm·cm^{-3}), compared to control (0.85 cm·cm^{-3}). Additionally, the bacterial inoculant IUNG has significantly decreased the RLD in roots classes between 0.2–0.5 mm in the most humid year.
3. The impact of inoculants on roots parameters was strongly related to weather conditions. In the dry year, a significant decrease of MRD, SRL and increase of RDM were observed.

4. The foliar fertilizer had a significant influence on the RLD and MRD values only within inoculant combination. Significantly higher RLD (1.05) accumulation of nitrogen in the plant and in the soil was found.

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